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V. Cyanide

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This is a review of the scientific literature on the biological and environmental effects of cyanide. Included in the review are a general summary and a comprehensive discussion of the following topics as related to cyanide and specific cyanide compounds: physical and chemical properties; occurrence; synthesis and use; analytical methodology; biological aspects in microorganisms, plants, wild and domestic animals, and humans; distribution, mobility, and persistence in the environment; assessment of present and potential health and environmental hazards; and review of standards and governmental regulations. More than 500 references are cited.

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FOREWORD

A vast amount of published material is accumulating as numerous research investigations are conducted to develop a data base on the adverse effects of environmental pollution. As this information is amassed, it becomes continually more critical to focus on pertinent, well-designed studies. Research data must be summarized and interpreted in order to adequately evaluate the potential hazards of these substances to ecosystems and ultimately to public health. The Reviews of the Environmental Effects of Pollutants (REEPs) series represents an extensive compilation of relevant research and forms an up-to-date compendium of the environmental effect data on selected pollutants.

Reviews of the Environmental Effects of Pollutants: V. Cyanide includes information on chemical and physical properties; pertinent analytical techniques; transport processes to the environment and subsequent distribution and deposition; impact on microorganisms, plants, and wildlife; toxicologic data in experimental animals including metabolism, toxicity, mutagenicity, teratogenicity, and carcinogenicity; and an assessment of its health effects in man. The large volume of factual information presented in this document is summarized and interpreted in the final chapter, "Environmental Assessment," which presents an overall evaluation of the potential hazard resulting from present concentrations of cyanide in the environment. This final chapter represents a major contribution by James L. Way from Washington State University.

The REEPs are intended to serve various technical and administrative personnel within the Agency in the decision-making processes, i.e., in the development of criteria documents and environmental standards, and for other regulatory actions. The breadth of these documents makes them a useful resource for public health personnel, environmental specialists, and control officers. Upon request these documents will be made available to any interested individuals or firms, both in and out of the government. Depending on the supply, the document can be obtained directly by writing to:

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ABSTRACT

This study is a comprehensive, multidisciplinary review of the health and environmental effects of cyanide and specific cyanide derivatives. Over 500 references are cited.

Cyanide production in the United States was about 700 million pounds in 1975, most of which was used for acrylonitrile production. The most important applications of inorganic cyanides are electroplating and metal treatments. Improper storage, handling, and disposal account for isolated instances of cyanide release to the environment. Tobacco smoke is probably one of the major sources of cyanide exposure to the general public.

Cyanide is a general respiratory poison acting by inhibition of cytochrome oxidase. Uptake occurs through inhalation, ingestion, or skin absorption. Because of the known dangers of cyanide, accidental acute poisonings are uncommon. Evidence of exposure to low levels of cyanide over prolonged periods is not well recognized. There is little data concerning the carcinogenic, teratogenic, and mutagenic properties of cyanide or the distribution and transformation of cyanides in air, land, or water.

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SECTION 1

SUMMARY

1.1 PROPERTIES AND ANALYSIS

Cyanides are defined as organic or inorganic compounds which contain the -C≡N grouping. Hydrogen cyanide (HCN) is a colorless liquid which boils at 25.7°C and freezes at -13.2°C; the gas is lighter than air and diffuses rapidly (Section 2.2.1). Free HCN is very reactive and occurs only rarely in nature; it is usually prepared commercially from NH_3 and CH_4 at elevated temperatures with a platinum catalyst. In aqueous solution, HCN is a weak acid with the ratio of HCN to CN^- being about 100 at pH 7.2, 10 at pH 8.2, and 1 at pH 9.2. Both HCN and cyanide salts form complexes with metals; this property is responsible for the industrial utility of these compounds. Cyanides from industrial activity are released mainly to water and, to a lesser extent, to the atmosphere. The degradation of cyanides through waste management procedures usually produces carbonates and nitrogen gas (Section 2.2.1.4).

Cyanide complexes a variety of metals, especially those of the transition series (Section 2.2.8). Ferricyanides and ferrocyanides have a variety of industrial uses but do not release free cyanide unless exposed to ultraviolet light. Thus, sunlight can lead to the formation of cyanide in wastes containing ferricyanides and ferrocyanides.

Cyanogen [$(CN)_2$] is an extremely poisonous, flammable gas which has a vapor pressure of about 5 atm at 20°C. It is prepared industrially from HCN and oxygen at 300 to 600°C with a silver catalyst and is used chiefly in the chemical industry as a high-energy fuel and for organic syntheses. Cyanogen reacts slowly with water to form HCN, cyanic acid, and other compounds. It is extremely reactive and is probably rapidly degraded in the environment.

Cyanates contain the -OCN radical. Inorganic cyanates, which are formed by oxidation of cyanide salts, are reactive and hydrolyze in water to form NH_3 and bicarbonate. Alkyl cyanates trimerize readily to form cyanurates. Alkyl isocyanates contain the -NCO radical and are formed from cyanates. They, too, are reactive; however, they are insoluble in water.

Thiocyanates (-SCN) are formed from cyanides and sulfur-containing materials and are more stable than cyanates (Section 2.2.4). Solutions of thiocyanates form HCN in acidic media; degradation of thiocyanate wastes is accomplished by procedures similar to those used for cyanide wastes.

Nitriles are defined as organic cyanides (RCN). They are easily prepared and exhibit a marked tendency to polymerize (Section 2.2.5). Most nitriles are fairly insoluble in water but are soluble in organic solvents. Acrylonitrile is an important raw material for the textile and rubber industries.

Cyanohydrins, $R_2C(OH)CN$, are toxic compounds which can decompose to HCN or CN^- under environmental conditions (Section 2.2.6). Calcium cyanamide (CaNCN) is commonly used as a fertilizer that reacts in the soil to yield urea.

Suitable analytical procedures are important for the detection of cyanide within the environment. Preparation of aqueous samples usually involves alkalizing the sample, removing interfering sulfides by precipitating and filtering lead sulfide, and removing interfering fatty acids by extracting the acidified aqueous phase with isooctane, hexane, or chloroform (Section 2.3.1). Additional steps may be necessary to remove other interfering substances. The sample can then be analyzed by one of several procedures (Section 2.3.2). Absorption spectrophotometry is probably the most widely used technique for determining cyanide concentrations of 1 mg/liter or less; modifications allow detection of cyanide down to 5 μg/liter. Speedy analyses and little sample preparation are attributes of the cyanide ion-selective electrode; the silver iodide membrane electrode is useful in the 10^{-3} to 10^{-5} *M* range. Indirect atomic absorption spectrometry is adequate for cyanide detection but is not extensively used. Fluorometry has a low detection limit but is presently limited in application. Gas chromatography is a sensitive procedure for detecting cyanide, usually by converting it to a cyanogen halide.

1.2 ENVIRONMENTAL OCCURRENCE

Cyanide production in the United States was about 700 million pounds for 1975; about 52% was used for acrylonitrile production, 18% for methyl methacrylate production, 14% for adiponitrile production, 7% for sodium cyanide production, and 9% for a variety of uses (Section 7.2). Inorganic cyanides have many uses; the two most important applications are electroplating and metal treatments (Table 7.1). Hydrogen cyanide is used as a rodenticide and an insecticide. Organic cyanides, such as acrylonitrile, are used for the production of acrylic and modacrylic fibers, nitrite elastomers, and plastics.

Industries concerned with the production and use of cyanide compounds generate wastes which contain large amounts of cyanide; for example, electroplating wastes contain 0.5% to 20% cyanide (Section 7.3). Paint manufacture and use, the steel industry, and mining operations all produce wastes with a high cyanide content. Fortunately, the toxicity of cyanides is well known. Waste management procedures are standard practice and remove most of the cyanide. Three standard procedures for cyanide destruction are alkaline chlorination (most common), electrolytic decomposition, and ozone oxidation (Sections 2.2.1.4 and 7.5). Cyanide can also be converted to less toxic compounds such as cyanate and ferricyanide. Improper storage, handling, and disposal account for isolated instances of cyanide release to the environment.

Data on the distribution of various cyanide compounds within the environment and on their transformations are sparse. Cyanides are not adsorbed or retained within soils (Section 7.4.1). Microbial metabolism apparently can rapidly degrade cyanide to CO_2 and NH_3 and thus eliminate any soil accumulation problem. Under anaerobic conditions, cyanides are converted by microbes to gaseous nitrogen compounds which escape to the atmosphere.

Cyanides are uncommon in U.S. water supplies. Small quantities of hydrocyanic acid, cyanide salts, or complexed cyanides sometimes occur (Section 7.4.2); however, they usually do not exceed the recommended maximum limit of 10 ppb. Volatile cyanides are not usually detected in the atmosphere (Section 7.4.3).

1.3 BIOLOGICAL ASPECTS IN MICROORGANISMS

A wide variety of microorganisms are able to metabolize cyanide. These organisms may play a role in the treatment of cyanide wastes. If a mixed population in a sludge process has not been exposed to cyanide, small cyanide concentrations (e.g., 0.3 ppm cyanide) can be toxic (Section 3.2.2.2.1). The population can become acclimated to cyanide, after which they are able to degrade wastes with higher cyanide concentrations. The presence of cyanide will affect the population structure in mixed population systems.

Several species of fungi are able to take up and metabolize cyanide (Section 3.2.2.2.2). The products of cyanide metabolism vary. *Fusarium solani* degrades cyanide to CO_2 and NH_3, whereas *Rhizoctonia solani* converts cyanide to α-aminobutyronitrile. The nitrile group of α-aminobutyronitrile is then hydrolyzed to give a α-aminobutyric acid. An unidentified basidiomycete can convert HCN to 4-amino-4-cyanobutyric acid, which is then hydrolyzed to glutamate. Thus, cyanide nitrogen is eventually released to the environment as NH_3 and cyanide carbon is released as CO_2.

Bacterial species which metabolize cyanide have also been isolated, but often the details of their metabolism have not been described (Section 3.2.2.2.3). *Chromobacterium violaceum* can apparently produce β-cyanoalanine and γ-cyano-α-aminobutyric acid from cyanide. Subsequent hydrolysis of the nitrile groups in these products returns the cyanide nitrogen atom to the environment as NH_3. Several species of bacteria form thiocyanate from cyanides and thiosulfates.

Microbes are also able to synthesize cyanide (Section 3.2.2.1). The biochemistry of cyanide production is not completely known, however. In some fungi, cyanide production is associated with autolysis, whereas in others it is formed throughout the growth cycle. Various bacteria produce cyanides. *Chromobacterium violaceum* can synthesize HCN from glycine. Again, some reports have suggested that cyanide can be synthesized by growing cultures, while others report that cyanide is synthesized from nonproliferating cells.

Cyanides are toxic to a wide range of microorganisms, but quantitative data are sparse (Sections 3.3.1 and 3.3.2). *Microregma heterostoma* (protozoa) and *Scenedesmus quadricauda* (alga) are very sensitive, having toxicity thresholds of 0.04 and 0.16 ppm CN^-, respectively (Section 3.3.1.1). Some bacteria are killed by exposure to 1 ppm cyanide; however, some other microorganisms have much higher tolerances. One gram-negative bacterium can tolerate up to 50 ppm cyanide; the fungi *Aspergillus* sp. and *Rhizopus nigricans* can tolerate up to 100 and 200 ppm cyanide, respectively. Besides decreased growth, the effects of exposure to cyanide have been reported to include altered cell morphology, decreased motility, and mutagenicity. Some microorganisms have become essentially resistant to cyanide.

Inhibition of respiration is an extensively studied effect of cyanide. In addition to the cyanide-sensitive electron transport pathway, many microbes have a cyanide-insensitive pathway. Examples are more common and better studied in the bacteria and fungi but are also known in algae and protozoa.

Cyanide inhibits DNA replication in *Escherichia coli* and perhaps DNA-repair processes in *Chlamydomonas reinhardi*. Amino acid transport in bacteria is also inhibited by cyanide. The above inhibitions were observed with 0.5 to 17 m*M* KCN. Cyanide also inhibits various microbial enzymes such as β-hemolysin, nitrite reductase, and nitrate reductase.

In summary, data exist concerning the cyanide inhibition of growth, respiration, and other physiological processes in isolated microbial systems, but there are virtually no data on the effects of cyanide exposure on natural microbial communities in soil and water. An exception is the mixed microbial populations in sewage sludge. Cyanide exposure most likely occurs sporadically and in low concentrations. Many microbes metabolize cyanide and many others synthesize it; however, the integration of these processes into the ecology of a given community is unknown.

1.4 BIOLOGICAL ASPECTS IN PLANTS

Free cyanide is not found in higher plants. Although the supporting data are sparse, plants can actively release HCN to the environment (Section 4.2.2). The ecological implications of such a release are unknown. Many plants, if not all, have the ability to metabolize administered cyanide (Section 4.2.1). A major pathway is the reaction of cyanide with cysteine to form β-cyanoalanine, which is then hydrolyzed to asparagine.

Other conversions of β-cyanoalanine occur in some species. Some lathyrogenic compounds, found in the genera *Lathyrus* and *Vicia*, are formed from β-cyanoalanine (Section 4.2.5). The lathyrogens are products of normal plant metabolism and do not indicate cyanide exposure. The origin of the endogenous cyanide radical in the lathyrogenic plants is unknown.

The cyanide radical is found in a variety of naturally occurring plant compounds. These compounds include cyanogenic glycosides, glycosides, lathyrogenic compounds, indoleacetonitrile, and cyanopyridine alkaloids.

Plants that contain cyanogenic glycosides are potentially poisonous because bruising or incomplete cooking can lead to hydrolysis of the glycoside and release of HCN (Section 4.2.3). There are about 20 major cyanogenic glycosides, of which only one or two occur in a given species. They are synthesized from amino acids and sugars and are found in a variety of economically important plants including sorghum, flax, lima bean, cassava, and many of the stone fruit. Their functions and further metabolism within the plant are unknown. Glucosinolates (thioglucosides) are also common secondary plant products and are formed in members of the Cruciferae (Section 4.2.6). Upon injury to these plants, the glucosinolates can be hydrolyzed to organic nitriles, isothiocyanates, or thiocyanates, depending on the pH. These compounds can produce human maladies (Section 6.3.3). The remaining natural products which contain cyanide groupings are of lesser importance and are restricted to a few taxa.

Nitrile herbicides are used in a variety of situations; all apparently act as metabolic inhibitors or uncouplers of electron flow (Section 4.3.4.5). They are used either as a preemergent herbicide (dichlobenil) or as a postemergent, contact herbicide (ioxynil and bromoxynil). Their metabolism is not well characterized, but they do not release cyanide (Section 4.2.9).

The major effect of cyanide on plants is the inhibition of respiration, which occurs through the inhibition of cytochrome oxidase (Section 4.3.1). Inhibition is not always complete, however, and the remaining activity is referred to as cyanide-insensitive respiration. Insensitive respiration varies with physiological status and pretreatment and is apparently due to electron flow through an alternate pathway which is not inhibited by cyanide. Inhibition of enzymes other than cytochrome oxidase is usually a result of (1) complexing of a metal cofactor, (2) reaction with carbonyl groups, or (3) reaction with disulfide bonds (Section 4.3.3).

Without respiration and the resulting ATP, a variety of other physiological processes, including ion transport and translocation, fail. Interestingly, the early stages of germination are stimulated by cyanide exposure, presumably due to the more extensive use of the pentose phosphate pathway during this period (Section 4.3.4.1). Cyanide produces chromosomal aberrations in some plants, but the mechanism of this action is unknown (Section 4.3.4.4).

Hydrogen cyanide has been used in greenhouses, in the field, and in commercial fumatoria as a fumigant to control insect populations (Section 4.3.4.6). This use can lead to cyanide residues in treated foods. The chemical forms of these residues are not known, but maximum residue limits are established for various foods.

1.5 BIOLOGICAL ASPECTS IN HUMANS AND EXPERIMENTAL ANIMALS

1.5.1 Metabolism

1.5.1.1 Uptake — Intake of cyanide can be by inhalation, ingestion, or absorption through the skin (Section 6.2.1). Whatever the route, cyanide is readily absorbed into the bloodstream and carried throughout the body. Death soon after exposure to lethal concentrations of cyanide is evidence of rapid absorption. Cyanide rapidly appears in the blood after inhalation of both HCN and cyanide salt dusts. Cyanogen, a gas at room temperature, releases hydrogen cyanide upon hydrolysis and is, therefore, toxic; no absorption values were available. When inhaled, the toxicity of halogenated cyanogens is comparable with that of HCN, but this should not infer toxicity is due to the cyano moiety. Some nitriles may also represent an inhalation hazard.

Ingestion of cyanides also causes the rapid appearance of cyanide in blood. Cyanogenic glycosides found in some plant foodstuffs release hydrogen cyanide during digestion, but release is slow and appearance of symptoms is delayed. Food in the stomach tends to delay absorption of cyanides. Hydrogen cyanide in liquid or vapor form can be absorbed through the skin; absorption is more rapid if the skin is cut, abraded, or moist.

1.5.1.2 Transport and Distribution — Once cyanide is in the bloodstream, it is distributed to other body tissues. Some cyanide binds to methemoglobin in the blood (Section 6.2.2.1). This binding is reversible and cyanide is released to plasma when the free cyanide concentration in the blood drops. After the initial exposure to cyanide, levels of cyanide in tissues other than blood increase considerably.

Cyanate reacts irreversibly with free sulfhydryl and amino groups of blood proteins to form carbamyl derivatives. Sodium nitroprusside is reported to react nonenzymatically with hemoglobin in red blood cells to form cyanide. Cyanogen chloride (CNCl) is also rapidly converted to HCN in the presence of red blood cells.

1.5.1.3 Detoxification — Most cyanide reactions, including those responsible for toxicity and for detoxification, occur within cells. Inhibition of cytochrome oxidase is probably the major reaction causing cyanide toxicity. The major detoxification pathway is the reaction of cyanide with thiosulfate in the presence of the enzyme, rhodanese, to produce thiocyanate (Section 6.2.3.1). Most tissues contain rhodanese; liver, kidney, brain, and muscle have higher levels than other tissues. Rhodanese is found within mitochondria. The content of the other reactant, thiosulfate, is low in the body; added thiosulfate can increase the LD_{50} for cyanide; thus, sulfur donors may be the limiting substrate for cyanide detoxification.

Minor detoxification pathways also exist. Cyanide combines with cystine to produce 2-aminothiazoline-4-carboxylic acid or 2-iminothiazolidine-4-carboxylic acid. This last compound is inert and is excreted. Cyanide also is believed to be metabolized through formic acid to form CO_2. In addition, cyanocobalamine ($CN-B_{12}$), a cyanide-containing form of vitamin B_{12}, is formed after cyanide exposure. However, the amounts of hydroxocobalamin (B_{12}) in the liver are sufficient to detoxify large doses of cyanide. Injection experiments in humans suggest that $CN-B_{12}$ is not metabolized; however, contradictory evidence also exists. Vitamin B_{12} given to mice shortly before or after cyanide reduces the toxicity of cyanide. The binding of cyanide by methemoglobin in blood also reduces free cyanide concentrations.

1.5.1.4 Excretion — Studies with mice and guinea pigs show that 1% to 2% of injected HCN can be eliminated by exhalation prior to death. However, most cyanide is metabolized and excreted in the urine as thiocyanate (Section 6.2.4). Tobacco smoke contains cyanide; the urine and body fluids of smokers contain higher concentrations of thiocyanates than those of nonsmokers. Levels of thiocyanate in urine increased from 3.7 mg/liter for people smoking 10 cigarettes per day to 20.0 mg/liter for people smoking 40 cigarettes per day. Cyanocobalamin is also excreted in the urine.

1.5.2 Effects

1.5.2.1 Mechanism of Action — Cyanide inhibits enzymatic activity by binding to the metallic cofactor in metalloenzymes (Section 6.3.1). Cytochrome oxidase, the most sensitive enzyme known, is completely inhibited by 10^{-8} moles/cm^3 of cyanide. Oxygen cannot then be utilized and cytotoxic anoxia occurs. Death results from depression of the central nervous system (CNS), the tissue most sensitive to anoxia. Cyanide in higher concentrations also inhibits other heme enzymes (catalase and peroxidase) as well as nonheme metalloenzymes (tyrosinase, ascorbic acid oxidase, and phosphatase).

1.5.2.2 Acute Effects — The effects of cyanide depend on the degree and rate of production of histotoxic hypoxia (Section 6.3.2). Fatal doses produce a brief stage of CNS stimulation and then depression followed by hypoxic convulsions and death. At 2000 ppm HCN in air, the first breath brings immediate deep, rapid breathing with collapse, convulsions, and cessation of breathing occurring within a minute. The total amount of HCN absorbed in rapid death may be as low as 7 mg/kg body weight. For HCN absorbed through the skin the LD_{50} is about 100 mg/kg body weight. Ingestion of KCN or NaCN produces similar effects but over a longer time (5 to 20 min after ingestion). About 3.5 mg HCN per kilogram body weight is absorbed when death occurs after NaCN or KCN ingestion.

Nitriles may first exert a pharmacologic action due to the entire molecule, but if cyanide is released from the nitrile faster than the cyanide can be detoxified, cyanide toxicity symptoms may occur.

1.5.2.3 Chronic Effects — Because of the known dangers of cyanide, accidental acute poisonings are uncommon. Exposure to low levels of cyanide over prolonged periods produce symptoms that differ from acute exposure and are not well recognized (Section 6.3.3).

Recently, chronic cyanide uptake has been correlated with diseases such as tobacco amblyopia, retrobulbar neuritis in pernicious anemia, Leber's optic atrophy, and Nigerian nutritional neuropathy. Defects in cyanide detoxification processes may play contributing roles. The neuropathies may result from demyelination of nerves in the CNS caused by cyanide-induced anoxia. Cyanide exposure can lead to pathology of the CNS in experimental animals. Tropical neuropathies in humans are characterized by optic atrophy, nerve deafness, and sensory spinal ataxia (Section 6.3.3.1). Chronic cyanide intoxication has been implicated by some reports in these diseases as well as in tropical amblyopia. Intake may result from the use of cassava, a cyanogenic plant, as a staple in the diet. Patients with these neuropathies have elevated plasma levels of thiocyanate and vitamin B_{12}. Other factors may contribute to the symptoms. For instance, a deficiency of sulfur in the diet may decrease the cyanide-to-thiocyanate detoxification process and, thus, increase the severity of the chronic symptoms.

Cyanide and thiocyanate may also be involved in endemic goiter and cretinism (Section 6.3.3.2). Again, the relationship occurs in tropical areas where cassava is a food staple. Thiocyanate, produced from detoxification of cyanide, has antithyroid activity and could be a factor, along with iodine deficiency, in goiter.

1.5.2.4 Carcinogenesis, Teratogenesis, and Mutagenesis — There is a paucity of information concerning the carcinogenic, teratogenic, and mutagenic properties of cyanide, and the information that is available warrants further investigation.

β-Aminopropionitrile (BAPN) is teratogenic, but this behavior is apparently a function of the whole molecule. Cleft palate was produced in 98% of the offspring of rats fed 270 mg BAPN on day 15 of the gestation period.

1.6 BIOLOGICAL ASPECTS IN WILD AND DOMESTIC ANIMALS

Since cyanide is a general respiratory poison acting by inhibition of cytochrome oxidase, acute exposure of aerobic organisms to cyanide may result in a histotoxic anoxia. Uptake, absorption, transport, distribution, and acute toxic effects in mammals are similar to that described for human and experimental animals in Section 1.5. Acute poisoning may occur when animals graze on cyanogenic plants such as sorghum and Sudan grass. Chronic cyanide poisoning also has been reported in grazing animals. Grazing on cyanogenic plants has been reported to induce sulfur deficiency in sheep, presumably because the sulfur is used to detoxify the released cyanide. In the rainy season, sheep feeding on the cyanogenic, low iodine plant *Cynodon plectostachyum* exhibit hypothyroidism. In this latter case, the hypothyroidism may be due to low dietary iodine, release of cyanide, or a combination of both factors.

Fish are the other major group of animals for which some cyanide data are available. Data on uptake, absorption, and excretion were few. Absorption probably occurs through gills and the gastrointestinal tract. Toxicity data suggest that absorption is not a limiting factor. In one field study, cyanide concentrations of 0.05 to 0.1 ppm were lethal to most game fish. Toxicity varies with species, physiological conditions, and with water pH, temperature, and content of oxygen and other solutes. Toxicity of cyanide is reduced by complexation with some cations such as iron and nickel. Generally, lowered oxygen concentrations in water or an increase in temperature increases the toxicity of a given concentration of cyanide. There is surprisingly little information on the interactions of cyanide with birds.

1.7 CONCLUSIONS

1. The major cyanide compounds of environmental interest are hydrogen cyanide, cyanide salts, and nitriles.

2. Cyanide forms stable complexes with most of the transition metals; however, cyanide can be released from some iron complexes with ultraviolet light.

3. Absorption spectrophotometry and volumetric titrimetry are two useful techniques for cyanide detection.

4. No exact numbers are available for current cyanide production in the United States, but production for 1976 was estimated to be about 700 million pounds. Most of this amount (52%) was used for acrylonitrile production.

5. Wastes generated from the metal plating and finishing industry contain high concentrations of cyanide; however, cyanide removal from these wastes is accomplished mainly by alkaline chlorination, electrolytic decomposition, or ozone oxidation.

6. Improper storage, handling, and disposal account for most instances of environmental cyanide release.

7. Tobacco smoke is probably one of the major sources of cyanide exposure to the general public.

8. Little is known about the distribution and transformation of cyanides in air, land, or water. Cyanides are not usually found in air and are not retained in soils. The recommended maximum concentration of cyanide in water (10 ppb) is seldom found to be exceeded.

9. Cyanide is a relatively reactive compound that is not found to accumulate in the environment. Various microbes are able to degrade cyanide to carbon dioxide and ammonia. Most other organisms can also metabolize or detoxify cyanide. Cyanide probably does not bioaccumulate nor biomagnify in food chains.

10. Although some bacteria, fungi, and a few higher plants are able to synthesize cyanide, the impact of this release on the environment is not known.

11. Cyanogenic glycosides, which occur in a variety of food and crop plants, may release cyanide only during destruction of the plant (i.e., crushing, bruising, or ingestion). This release is not known to be a source of lethal quantities of cyanide to humans, but in tropical areas where these plants form a major dietary staple, chronic cyanide exposure has been proposed.

12. Inhibition of aerobic respiration is the best-studied effect of cyanide on aerobic organisms. The lethal dose varies with species. In humans, death results from depression of the central nervous system, the tissue most sensitive to anoxia.

13. Uptake of cyanide by animals occurs through inhalation, ingestion, or skin absorption. Absorption readily occurs and cyanide is rapidly distributed by the blood system to other body tissues.

14. Some cyanide reacts with methemoglobin to form cyanmethemoglobin. The major mammalian detoxification mechanism is attributed to the rhodanese-catalyzed reaction of cyanide with a sulfur donor to produce thiocyanate, which is then excreted in the urine. Some alternative detoxification pathways also exist.

15. Accidental acute cyanide poisoning occurs but is uncommon in humans.

16. Chronic human effects are not well recognized and they have been implicated in the tropics. Diseases such as amblyopia, retrobulbar neuritis, Leber's optic atrophy, and various neuropathies occur in tropical areas where cyanogenic plants (e.g., cassava) form a large portion of the diet. Endemic goiter and cretinism may be partially related, in some cases, to chronic cyanide exposure.

17. β-Aminopropionitrile apparently is teratogenic in some mammals.

18. Little information exists on the metabolism and effects of cyanide on other groups of organisms (birds, amphibians, reptiles, and invertebrates).

19. Fish are sensitive to cyanide; sensitivity varies with species, physiological condition, pH, temperature, oxygen level, and occurrence of other compounds in the water. Cyanide concentrations greater than 0.1 ppm are lethal to many species.

SECTION 2

CHEMICAL AND PHYSICAL PROPERTIES AND ANALYSIS

2.1 SUMMARY

Cyanides comprise a group of organic and inorganic compounds containing the CN radical. Many of these compounds are extremely useful in industry. The widespread usefulness of cyanides stems from the tendency of these compounds to form strong complexes with most metals — particularly those of the transition series. This characteristic accounts for the use of cyanides in metallurgy, electroplating, and metal-cleaning operations.

The complex-forming tendency of cyanides is also responsible for their toxicity; cyanide ions form stable complexes with various metals in enzyme systems, interfering with their normal operation. In particular, the cyanide ion reacts with the trivalent iron present in mitochondrial ferricytochrome oxidase, preventing further oxidation and reduction reactions and causing a histotoxic anoxia. Detoxification of cyanide-complexed ferricytochrome oxidase requires either (1) reversal of the complexing reaction by the introduction of a competing chemical species, such as methemoglobin which contains trivalent iron, or (2) introduction of a compound which reacts with cyanide to form a less toxic product. An exogenous sulfur donor, sodium thiosulfate, is usually used to convert cyanide to a less poisonous species. The resulting thiocyanate is considerably less toxic than the cyanide ion. Toxicity of the various organic cyanides sometimes depends on whether cyanide ions are formed during their metabolism. In general, the lower aliphatic nitriles are more toxic than the purely aromatic nitriles. Solutions of ferrocyanides and ferricyanides have relatively low toxicity unless they are exposed to sunlight or ultraviolet radiation, in which case hydrogen cyanide and cyanide ions are formed. The nitroferricyanides are believed to dissociate in vivo to form cyanide ions and therefore may be toxic. Cyanogenic glycosides are natural compounds produced in a variety of plants. Although presumed to be relatively harmless in the pure state, these substances are capable of producing quantities of hydrogen cyanide when hydrolyzed.

Many chemical treatments for removal of cyanides from industrial wastewaters have been suggested; however, only three processes are sufficiently versatile and economical for widespread use. Alkaline chlorination, probably the most frequently used method, is adaptable to both large- and small-scale use. Electrolytic decomposition is effective for wastes containing high concentrations of cyanide but is not useful for processing dilute solutions. Ozone oxidation may be an economical alternative to alkaline chlorination for selected cyanide wastes, but it is not effective for all cyanide complexes.

A variety of good analytical methods is available for determining cyanide in environmental samples. Absorption spectrophotometry and volumetric titrimetry are the most widely used techniques, mainly because of their inherent methodical and technical simplicity and low cost. Ion-selective electrodes allow direct and sensitive measurements of cyanide in selected samples. Indirect atomic absorption spectrophotometric methods are useful for samples containing cyanide in the parts per million range. Fluorometry and gas chromatography can be sensitive methods for analyzing cyanide. The latter technique also can distinguish speciated cyanides.

2.2 PHYSICAL AND CHEMICAL PROPERTIES

Cyanides are compounds containing a characteristic group, the cyanide radical (CN). Many of these compounds have separate names which designate distinctive features of the cyanide group. The most important classifications are hydrogen cyanide, cyanogen, cyanate, isocyanate, thiocyanate, nitrile, isocyanides, cyanohydrin, cyanogenic compounds, and complex cyanide compounds. Pertinent physical and chemical properties of these groups are discussed in the following sections.

2.2.1 Hydrogen Cyanide and Salts

Hydrogen cyanide, a widely used industrial chemical known also as hydrocyanic acid, prussic acid, and HCN, was first prepared by Scheele in 1782.

2.2.1.1 Physical Properties — Hydrogen cyanide is a colorless, flammable liquid or gas which boils at 25.7°C and freezes at -13.2°C. It is miscible with water and alcohol but is only slightly soluble in ether (Stecher, 1968). The penetrating odor of hydrogen cyanide is reminiscent of bitter almonds. The gas is lighter than air (0.947 at 31°C, air = 1) and therefore rises and diffuses rapidly. Hydrogen cyanide polymerizes spontaneously and sometimes violently when it is not absolutely pure (Faith, Keyes, and Clark, 1965, p. 456). A small quantity (0.1% to 0.5%) of sulfuric or phosphoric acid is usually added to hydrogen cyanide to inhibit this reaction (Montgomery, 1965, p. 583). Other physical properties of hydrogen cyanide are listed in Table 2.1. The chief physical characteristics of sodium cyanide and potassium cyanide, the two most important salts of hydrogen cyanide, are given in Tables 2.2 and 2.3, respectively.

2.2.1.2 Chemical Properties — Hydrogen cyanide is a weak acid; in solutions at ordinary pH values, the molecular form, HCN, predominates. At 25°C the pH of HCN is 9.2 (Montgomery and Stiff, 1971). Thus, hydrogen cyanide is readily liberated from solutions of its salts when the solutions are treated with strong mineral acids. This behavior sometimes causes inadvertent gassings in chemical laboratories when waste cyanide and acid solutions are flushed through a common drain (Polson and Tattersall, 1969, p. 130). Hydrogen cyanide is effectively absorbed by bases such as alkali hydroxides and soda lime.

The widespread usefulness of hydrogen cyanide is related to the strong tendency of this compound and its salts to form complexes with many metals. For example, sodium cyanide is used in metallurgy for the

TABLE 2.1. PHYSICAL PROPERTIES OF HYDROGEN CYANIDE

Melting point	-13.24 °C
Boiling point	25.70 °C
d_4^t	
0 °C	0.7150
10 °C	0.7017
20 °C	0.6884
Specific gravity, aqueous solution, d_{18}^{18}	
10.04% HCN	0.9838
20.29% HCN	0.9578
60.23% HCN	0.829
Vapor pressure	
-29.5 °C	50.24 mm Hg
0 °C	264.39 mm Hg
27.2 °C	807.23 mm Hg
Vapor density at 31 °C (air = 1)	0.947
Surface tension at 20 °C	19.68 dynes/cm
Viscosity at 20.2 °C	0.2014 cP
Specific heat	
-33.1 °C	13.95 cal/mole
16.9 °C	16.94 cal/mole
Heat of fusion at -14 °C	1.72 kcal/mole
Heat of formation	
gas	-30.7 kcal/mole
liquid at 18 °C, 1 atm	-24.0 kcal/mole
Heat of combustion	159.4 kcal/mole
Critical temperature	183.5 °C
Critical density	0.195 g/ml
Critical pressure	55 kg/cm^2
Dielectric constant	
0 °C	158.1
20 °C	114.9
Dipole moment, gas, at 3 to 15 °C	2.1×10^{-8} esu
Dissociation constant, K_{18}	7.2×10^{-10}
Conductivity at 0 °C	$3.3 \times 10^{-6}\ \Omega^{-1}\ \text{cm}^{-1}$
Heat of vaporization	6027 cal/mole
Heat of polymerization	10.2 kcal/mole

Source: Adapted from Montgomery, 1965, Table 1, p. 575. Reprinted by permission of the publisher.

TABLE 2.2. PHYSICAL PROPERTIES OF SODIUM CYANIDE

Melting point	
100%	563.7 (±1) °C
98%	560 °C
Boiling point (extrapolated)	1500 °C
Density	
cubic	1.60 g/cm^3
orthorhombic	1.62 to 1.624 g/cm^3
molten, at 700 °C	1.22 g/cm^3 (approx)
Vapor pressure	
800 °C	0.76 mm Hg
900 °C	3.34 mm Hg
1000 °C	12.4 mm Hg
1100 °C	36 mm Hg
1200 °C	90 mm Hg
1300 °C	204 mm Hg
1360 °C	314 mm Hg
Heat capacity, 25 to 72 °C	0.33 cal/(g)(C)
Heat of fusion	75.0 cal/g
Heat of vaporization	761 cal/g
Heat of formation, ΔH_f^o, NaCN (c)	-21.46 kcal/g-mole
Heat of solution, ΔH_{soln} in 200 g-mole H_2O	+0.36 kcal/g-mole
Hydrolysis constant, K_h, 25 °C	2.51×10^{-5}

Source: Adapted from Mooney and Quin, 1965, Table 1, p. 586. Reprinted by permission of the publisher.

extraction of gold from ores and in electroplating baths because it forms stable soluble complexes of the type $Au(CN)_2^-$:

$$8NaCN + 4Au + O_2 + 2H_2O = 4NaAu(CN)_2 + 4NaOH.$$

Similar behavior makes alkali cyanide solutions excellent for cleaning silverware and other precious metals and is responsible for their general use in industry as metal cleaners (Arena, 1974, p. 210). The complexing nature of cyanides is also utilized in chemical synthesis and photography. Other aspects of the chemistry of cyanides have been reviewed in detail by Williams (1948).

2.2.1.3 Synthesis and Occurrence — Large quantities of hydrogen cyanide are synthesized in the United States (Section 7.2); Faith, Keyes, and Clark (1965, p. 455) estimated the production in 1963 at 136 million kilograms (300 million pounds). Although it can be manufactured from a variety of starting materials (e.g., coke-oven gas, calcium cyanide, or formamide),

TABLE 2.3. PHYSICAL PROPERTIES OF POTASSIUM CYANIDE

Melting point	
100%	634.5°C
96.05%	622°C
Density	
cubic at 20°C	1.553 g/cm^3
cubic at 25°C	1.56 g/cm^3
orthorhombic at -60°C	1.62 g/cm^3
Specific heat, 25 to 72°C	0.24 cal/(g)(C)
Heat of fusion	3.5 kcal/g-mole
Heat of formation, ΔH_f°	-26.90 kcal/g-mole
Heat of solution, ΔH_{soln}	+2.8 kcal/g-mole
Hydrolysis constant, 25°C	2.54 10^{-5}
Solubility in water at 25°C	71.6 g per 100 g H_2O

Source: Adapted from Mooney and Quin, 1965, Table 3, p. 597. Reprinted by permission of the publisher.

in most modern plants hydrogen cyanide is synthesized by passing ammonia, air, and natural gas over a platinum catalyst at an elevated temperature:

$$2NH_3 + 3O_2 + 2CH_4 \rightarrow 2HCN + 6H_2O.$$

The product is then concentrated by distillation, stabilized to prevent polymerization, and stored in cylinders (Faith, Keyes, and Clark, 1965, p. 454).

Free hydrogen cyanide occurs only rarely in nature because of the high reactivity of the molecule. However, the gas may sometimes be found in the atmosphere as a result of manufacturing operations, the incomplete combustion of nitrogen-containing materials (Polson and Tattersall, 1969, p. 130), or more often, the fumigation of ships, warehouses, or agricultural areas. Usually, the concentration of cyanide used for fumigation is <1%. Cylinders of liquid hydrogen cyanide are the most economical sources of gas for such work; however, for convenience, various absorption preparations are sometimes used [e.g., Zyklon (liquid hydrogen cyanide absorbed in fuller's earth), HCN discoids (liquid hydrogen cyanide absorbed in wood fibre discs), and Saftifume briquets (a mixture which produces hydrogen cyanide and cyanogen chloride from sodium cyanide and sodium chlorate mixed with sand)] (Jacobs, 1967, p. 723).

Hydrogen cyanide and its salts enter industrial waste streams from ore extracting and mining processes, synthetics manufacturing, coal-coking furnaces, photographic processing, and operations involving the electroplating of zinc, cadmium, and precious metals. The electroplating industry is considered a major source of cyanide waste (Watson, 1973) The cyanide content of natural waters, however, is usually low because of the reactivity of cyanide and the waste treatment procedures (Section 7.4.2).

2.2.1.4 Chemical Treatment of Cyanide Wastes — Many chemical treatments for removal of cyanide from industrial wastewaters have been suggested; however, only a few of these processes are sufficiently versatile and economical for widespread use. The current, most-used methods appear to be alkaline chlorination, electrolytic decomposition, and ozone oxidation (Watson, 1973, p. 147) (Section 7.5).

Alkaline chlorination involves the treatment of the waste cyanide solution with alkali and chlorine gas at room temperature. In most instances, the cyanide radical is completely disrupted and the carbon fragment is converted to carbonate and the nitrogen to nitrogen gas:

$$2NaCN + 5Cl_2 + 12NaOH \rightarrow N_2 + 2Na_2CO_3 + 10NaCl + 6H_2O.$$

Special equipment is required for the safe addition of chlorine, and some form of agitation is needed to obtain adequate mixing and reaction rates. The overall reaction is slow, usually requiring hours for completion, especially if the solution contains appreciable quantities of heavy metals. The process is better suited to the large-scale rather than the small-scale user because of the need for instrumentation to control the addition of reagents and the quality of the effluent.

Small-scale users can eliminate the problems of handling and metering chlorine gas by using solid hypochlorites such as sodium hypochlorite ($NaOCl$), calcium hypochlorite [$Ca(OCl)_2$], or bleaching powder ($CaOCl_2$). These compounds destroy cyanide without the addition of alkali:

$$2NaCN + 5NaOCl + H_2O \rightarrow N_2 + NaHCO_3 + 5NaCl,$$

$$4NaCN + 5Ca(OCl)_2 + 2H_2O \rightarrow 2N_2 + 2Ca(HCO_3)_2 + 3CaCl_2 + 4NaCl,$$

$$2NaCN + 5CaOCl_2 + H_2O \rightarrow N_2 + Ca(HCO_3)_2 + 4CaCl_2 + 2NaCl.$$

The process is relatively simple; although agitation is still required, it is only necessary to add the hypochlorite as a solution or as a solid to the wastewater. The reaction is more rapid with hypochlorites than with chlorine gas. However, the cost of the hypochlorite process is about twice that of the chlorine treatment (Watson, 1973, p. 158).

Electrolytic decomposition is frequently used to process waste containing high concentrations of cyanide. Usually, the waste solution is electrolyzed for 10 to 20 days at about 94°C. At first, cyanide is completely converted to carbon dioxide and ammonia; later, as the solution becomes more dilute, the reaction fails to go to completion and cyanate accumulates in the electrolyte. The cyanate is usually decomposed by an alternate process. The cyanide concentration of the waste solution after electrolysis usually varies from 0.1 to 0.4 mg/liter (Watson, 1973, p. 160). Obviously, this method is unsuitable for use with wastes containing low concentrations of cyanide.

Ozone may be used to replace chlorine in the treatment of some cyanide wastes. Solutions containing zinc, nickel, and copper cyanide complexes are readily processed by this reagent. The cobalt complex, however, is resistant to this treatment (Kandzas and Mokina, 1969). Ozonation was used at the Boeing Aircraft metal-working plant in Wichita, Kansas, to detoxify a 500-gpm stream containing 25 mg/liter cyanide. Complete oxidation of cyanide to cyanate was achieved with partial conversion of cyanate to final end products (Anonymous, 1958). Ozone also has been recommended for use in recovering or destroying complex cyanides present in commercial photofinishing waste solutions (Hendrickson and Daignault, 1973).

2.2.1.5 Chemical Basis for Toxicity — Cyanides are among the most rapid-acting poisons known (Goodman and Gilman, 1970, p. 934). The toxicity of these compounds is due to their dissociation into cyanide which complexes with the metals present in various enzymes, inhibiting their catalytic activity (Fassett, 1963, p. 1993) (Section 6.3.1). The specific reactions depend on unspecified physiological conditions and are not well characterized; however, the tendency of cyanide ions to form complex anions with transition metals, as shown by the following equations, is well established (Durrant and Durrant, 1962, p. 582). The indicated reactions may generally be considered simplified examples of more complex cellular chemistry:

$$NaCN \rightarrow Na^{+} + CN^{-},$$

$$6CN^{-} + Fe^{2+} \rightarrow [Fe(CN)_6]^{4-},$$

$$6CN^{-} + Fe^{3+} \rightarrow [F_6(CN)_6]^{3-},$$

$$6CN^{-} + Mn^{3+} \rightarrow [Mn(CN)_6]^{3-},$$

$$6CN^{-} + Co^{3+} \rightarrow [Co(CN)_6]^{3-}.$$

The reaction which is most sensitive to cyanide and which is believed to be predominantly responsible for toxicity involves the complexing of trivalent iron contained in mitochondrial cytochrome oxidase. This complexing prevents further oxidation and reduction reactions in the normal electron transport system and causes a histotoxic anoxia. Death from cyanide exposure is not due to inhibition of oxygen transport but to the inhibition of cytochrome oxidase and therefore, the failure of tissue utilization of oxygen, especially at the central nervous system (Section 6.3.1) (Arena, 1974, pp. 135-211).

2.2.1.6 Chemistry of Detoxification — In principle, it should be possible to reduce the toxicity of ingested cyanide by two different tactics: (1) supplying an alternate source of trivalent iron to compete with the ferri cytochrome oxidase for the available cyanide ion or (2) introducing a reagent which converts the cyanide ion to a less toxic form. In practice, both techniques are usually used. Obviously, implementation must be limited to rapid reactions which do not drastically upset the equilibrium of the patient. For example, sodium thiosulfate is the reagent usually chosen to implement the second technique. With the aid of the mitochondrial enzyme sulfurtransferase (rhodanese), this reagent reacts with the cyanide ion to form the relatively harmless thiocyanate ion, which is readily excreted in the urine (Goldstein, Aronow, and Kalman, 1974, p. 266):

$$CN^- + S_2O_3^{2-} \rightarrow CNS^- + SO_3^{2-}.$$

This enzymatic reaction occurs in vivo since sulfurtransferase is widely distributed in the tissues. This reaction is very rapid, but is incapable of handling massive doses of cyanide primarily due to substrate limitation of the sulfur donors (Gleason et al., 1969, p. 75). Additional discussion of cyanide detoxification in humans is found in Section 6.2.3.

2.2.2 Cyanogen and Cyanogen Halides

Cyanogen, the simplest compound containing the cyanide group, has the formula $(CN)_2$ (Table 2.4). It is a colorless, flammable gas which freezes at -27.9°C and boils at -21.2°C. Its vapor pressure at 20° is about 5 atm. Cyanogen is soluble in and slowly reacts with water to pro-

TABLE 2.4. PHYSICAL PROPERTIES OF CYANOGEN

Boiling point	-21.17°C
Melting point	-27.9°C
Critical pressure	59.6 atm
Critical temperature	128.3°C
Density of gas	2.321 g/liter
Density of liquid at boiling point	0.9537 g/ml
Dipole moment	0.38×10^{-18} esu
Heat of combustion	261.7-261.9 kcal/mole
Heat of dissociation	120-130 kcal/mole
Heat of formation (298.1°C, gas)	69.1-73.8 kcal/mole
Heat of vaporization at boiling point	0.5778 kcal/mole
Surface tension at boiling point	21.98 dynes/cm
Trouton's constant	22.94

Source: Adapted from Brotherton and Lynn, 1959, Table 1, p. 843. Reprinted by permission of the publisher.

duce hydrogen cyanide, cyanic acid (HOCN), and other compounds (Kleinberg, Argersinger, and Griswold, 1960, p. 356). The gas has a latent energy comparable to acetylene; mixtures of cyanogen and oxygen are explosive. It is used in the chemical industry as a high-energy fuel (Brotherton and Lynn, 1959). Cyanogen and its halide derivatives are also used in organic synthesis, as fumigants and pesticides, and in gold-extraction processes. It is also encountered as a rocket or missile propellant, in processes which involve heating of nitrogen-containing organic compounds, and in the exhaust gases of blast furnaces (Fishbein, 1973).

Cyanogen is extremely reactive; it does not long remain unchanged in the environment after introduction. Cyanogen and its halide derivatives are extremely poisonous; their toxicity is comparable to hydrogen cyanide, probably because of their conversion of the latter in the body (Fishbein, 1973, p. 361):

$$(CN)_2 + H_2O = HCN + HOCN,$$

$$CNCl + H_2O = HCN + HOCl.$$

Cyanogen is most conveniently prepared in the laboratory by the addition of potassium cyanide to copper sulfate:

$$2CuSO_4 + 4KCN \rightarrow Cu_2(CN)_2 + 2K_2SO_4 + (CN)_2.$$

Commercial production is based on a more efficient reaction in which hydrogen cyanide is oxidized at 300°C to 600°C with air and a silver catalyst:

$$4HCN + O_2 \text{ (air)} \rightarrow 2(CN)_2 + 2H_2O.$$

Cyanogen halides are formed when an aqueous solution of hydrogen cyanide or mercuric cyanide is treated with the free halogen. They are also obtained by the action of a mixture of bleaching powder and a halide acid on sodium cyanide (Williams, 1948, p. 6). Cyanogen chloride is a colorless, volatile liquid which boils at 13.8°C and freezes at -6°C. It is soluble in water, alcohol, and ether (Stecher, 1968). The vapor is highly irritating; its toxicity is like that of hydrogen cyanide (Section 6.2.2.1). The properties of the bromide are similar.

The chemistry of cyanogen and its derivatives has been reviewed extensively by Brotherton and Lynn (1959) and by Williams (1948).

2.2.3 Cyanates and Isocyanates

Inorganic cyanates are compounds containing the radical -OCN; they are formed when cyanides are treated with mild oxidizing agents (Norbury, 1975):

$$KCN + O_2 \text{ (air)} \rightarrow KOCN.$$

Crystalline alkali cyanates are colorless or white stable solids, but aqueous solutions of these compounds readily hydrolyze to ammonia and the corresponding bicarbonate. Some physical properties of these salts are shown in Table 2.5. Sodium and potassium cyanates, when compared to cyanide are relatively nontoxic to humans and animals (Arena, 1974, p. 164). However, the use of cyanate in the treatment of sickle cell anemia (Cerami, 1974) did induce various toxic lesions such as cataracts (Nicholson et al., 1976) and polyneuropathy (Peterson et al., 1974). Details of metabolism are unknown, but it is presumed that the toxic effect is caused by the cyanate ion per se and not by breakdown products (Fassett, 1963, p. 2034). Because of their relative instability and low toxicity, inorganic cyanates probably pose few environmental problems. Alkyl cyanates can be prepared by the action of sodium alkoxide on a cyanogen halide, but these esters usually trimerize immediately to form the cyanurate (Sidgwick, 1966, p. 462). Cyanates are encountered chiefly in manufacturing operations, especially the preparation of organic compounds (Zuzik, 1972).

TABLE 2.5. PHYSICAL PROPERTIES OF SOME CYANATES

Cyanate	Formula	Melting point (°C)	Specific gravity	Solubility		
				Water	Ether	Benzene
Ammonium	NH_4OCN	Decomposes, 60	1.34	Very soluble	Slightly soluble	
Lead	PbOCN	Decomposes		Slightly soluble		
Potassium	KOCN	315	2.0	Decomposes (hot)	Soluble	
Silver	AgOCN	Decomposes	4.00	Soluble (hot)		
Sodium	NaOCN	550	1.94	Soluble	Slightly soluble	Slightly soluble

Source: Adapted from Zuzik, 1972, pp. 937-940.

Potassium cyanate reacts with dialkyl sulfate to form alkyl isocyanates which have the general formula RNCO. These volatile liquids are very reactive, have a pungent odor, are insoluble in water, but are soluble in acetone, ethyl acetate, toluene, and kerosene. Alkyl isocyanates are widely used in the production of polyurethane plastics, foams, fibers, and surface coatings. Two of these compounds, toluene diisocyanate and diisocyanatodiphenyl methane, are known to cause asthmatic reactions in sensitized subjects (Morgan and Seaton, 1975). The mechanism of interaction is unclear, but isocyanates are known to react with free amino groups in proteins; they should thus be capable of forming antigens (Fassett, 1963, p. 2033).

2.2.4 Thiocyanates

Compounds containing the radical SCN are known as thiocyanates; they are formed by treating cyanides with sulfur or sulfur-containing reagents (Latimer and Hildebrand, 1951, p. 298):

$$KCN + S \rightarrow KSCN.$$

Thiocyanates are frequently encountered in the environment; they are widely used in printing and dyeing textiles, photography, analytical chemistry, insecticides, and in the manufacture of artificial mustard oil (Stecher, 1968).

2.2.4.1 Physical and Chemical Properties — Thiocyanates are more stable than cyanates; they form complexes with many elements, usually coordinating through the nitrogen atom to the first transition series metals and through the sulfur atom to the metals of the second and third transition series (Cotton and Wilkinson, 1962, p. 467). The alkali and alkaline earth thiocyanates are colorless, deliquescent salts which dissolve easily in water, alcohol, and acetone. Other physical properties of potassium thiocyanate are given in Table 2.6. Dry thiocyanate salts are decomposed on heating with concentrated sulfuric acid:

$$2KSCN + 6H_2SO_4 \rightarrow K_2SO_4 + (NH_4)_2SO_4 + 2CO + 2H_2O + 6SO_2,$$

but hydrogen cyanide is usually formed when acids are added to thiocyanate solutions, especially under oxidizing conditions (Williams, 1948, p. 258). Thiocyanate dissociates into cyanide and sulfate when electrolyzed:

$$KSCN + 3O + 2KOH \rightarrow KCN + K_2SO_4 + H_2O.$$

Potassium thiocyanate reacts with alkyl halides or alkyl hydrogen sulfate to form liquid esters which have a garliclike odor and varying degrees of toxicity. Several of these esters are used as insecticides (Dreisbach, 1971, p. 117).

2.2.4.2 Toxicity of Thiocyanates — Although small quantities of thiocyanates are normally present in human cells, presumably through the detoxification of dietary cyanides by the enzyme sulfurtransferase (Section 2.2.1.6), larger quantities cause chronic and acute poisoning (Arena, 1974, p. 600). The poisoning mechanism appears to involve the reaction of thiocyanate with oxyhemoglobin to yield sulfate and cyanide ions by the lacto-peroxidase-hydrogen peroxide system (Chung and Wood, 1971) and thiocyanate with sulfite to yield thiosulfate and cyanide ions (Goldstein and Rieder, 1951). The latter reaction is not the reverse of the sulfurtransferase (rhodanese) reaction described in Section 2.2.1.6 as it is attributed to the action of an enzyme, thiocyanate oxidase (Williams, 1959, p. 392).

2.2.4.3 Detoxification of Thiocyanate Wastes — The ready rupture of the sulfur-carbon bond occurring in reactions described in Section 2.2.4.1 indicates that methods normally used in detoxifying cyanide wastes can also be used effectively for wastes containing thiocyanate.

2.2.5 Nitriles and Isocyanides

Nitriles are organic cyanides with the general formula RCN. They can be prepared by many different chemical reactions such as the treatment of (1) potassium cyanide with alkyl sulfates or alkyl halides, (2) amides with a dehydrating agent, or (3) aldoximes with acetic anhydride.

TABLE 2.6. PHYSICAL PROPERTIES OF SOME IMPORTANT CYANIDES

Compound	Color	Formula	Formula wt (g)	Melting point (°C)	Boiling point (°C)	Density or specific gravity	Solubility		
							Water	Alcohol	Ether
Potassium thiocyanate	Colorless	KSCN	97	173.2	Decomposes 500	1.886 (14°C)	Soluble	Soluble	
Acetonitrile	Colorless	CH_3CN	41	-42	81.6	0.783 (25°C)	Miscible	Miscible	Miscible
Ethyl isocyanide		C_2H_5OCN	71		Decomposes 162	0.89 (20°C)	Insoluble	Miscible	Miscible
Lactonitrile	Colorless	$CH_3CH(OH)CN$	71	-40	182	0.9877 (20°C)	Miscible	Miscible	Soluble
Amygdalin		$C_{20}H_{27}NO_{11}$	457	223			Very soluble (hot)	Slightly soluble	Insoluble
Potassium ferricyanide	Red	$K_3Fe(CN)_6$	329	Decomposes		1.85 (25°C)	Soluble	Insoluble	
Potassium ferrocyanide	Yellow	$K_4Fe(CN)_6 \cdot 3H_2O$	422	$-3H_2O$, 70	Decomposes	1.85 (17°C)	Soluble	Insoluble	Insoluble
Sodium nitroferricyanide	Red	$Na_2(NO)Fe(CN)_5 \cdot 2H_2O$				1.72 (20°C)	Soluble	Soluble	

Source: Compiled from various sources.

One of the most important industrial compounds, acrylonitrile ($H_2C=CHCN$), is produced by the catalytic addition of hydrogen cyanide to acetylene and by the reaction of ammonia with propylene (National Academy of Sciences, 1975, p. 214). The simple aliphatic nitriles are liquids; higher members of this series are crystalline solids. Acrylonitrile, acetonitrile, propionitrile, and *n*-butyronitrile boil at 77.3, 81.6, 97, and 118°C, respectively. Nitriles have limited water solubility but are generally miscible in all proportions with most of the common organic solvents such as acetone, benzene, methanol, petroleum ether, and toluene. Other physical properties of important nitriles are given in Tables 2.6 and 2.7. There is a marked tendency for nitriles to polymerize; acrylonitrile polymerizes explosively in the presence of concentrated alkali. The tendency to polymerize is the basis for the use of acrylonitrile and adiponitrile in the manufacture of acrylic or nylon fibers and synthetic rubber. Nearly 550,000 tons of acrylonitrile were manufactured in seven plants in the United States during 1972 (National Academy of Sciences, 1975, p. 213). The occurrence of synthetic nitriles in the environment is largely due to losses associated with the production, transportation, and consumption of acrylonitrile and related compounds. In the United States, the annual loss of acrylonitrile is estimated to be about 7000 tons (National Academy of Sciences, 1975, p. 222).

TABLE 2.7. PHYSICAL PROPERTIES OF ACRYLONITRILE

Boiling point	77.3°C
Freezing point	-83.55°C
Specific gravity at 20°C	0.8060
Vapor density, air = 1	1.83
Viscosity at 24°C	0.34 cP
Surface tension at 24°C	27.3 dynes/cm
Flash point, open cup	0°C
Latent heat of vaporization at 25°C	7.8 kcal/mole
Latent heat of fusion at 25°C	36.20 kcal/mole
Refractive index at 25°C	1.3888
Water solubility at 20°C	5.35%
Solubility of water in acrylonitrile at 20°C	3.1%
Solubility in most organic solvents	Miscible

Source: Adapted from National Academy of Sciences, 1975, taken from outline on p. 209. Reprinted by permission of the publisher.

Isocyanides (formerly called isonitriles or carbylamines) are organic compounds which have the general formula RNC (Cahn, 1974, p. 102). The alkyl isocyanides are colorless liquids which have low molecular weight and distill without decomposition. The methyl, ethyl, and phenyl derivatives boil at 59, 79, and 166°C, respectively (Durrant and Durrant, 1962, p. 583). They are slightly soluble in water and readily soluble in alcohol and ether. Isocyanides possess a very disagreeable odor and are generally considered hazardous, although little research has been performed with them. Early investigators reported methylisocyanide to be more toxic than hydrogen cyanide and ethylisocyanide to be eightfold less so. It is uncertain if the usual antidotes for hydrogen cyanide are effective in treating poisoning by isonitriles (Fassett, 1963, p. 2031). Although known since 1868, isocyanides are little used outside the laboratory; they are not widely distributed in the environment.

2.2.6 Cyanohydrins

Cyanohydrins are organic compounds having a cyanide and a hydroxyl radical attached to a common carbon atom; they comprise a special class of nitriles whose general formula is $R_2C(OH)CN$. Cyanohydrins are prepared by treating carbonyl compounds with hydrogen cyanide or chlorohydrins with sodium cyanide (Fieser and Fieser, 1961, p. 418):

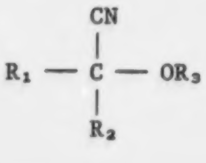
$$R_2CO + HCN \rightarrow R_2C(OH)CN,$$

$$R_2C(OH)Cl + NaCN \rightarrow R_2C(OH)CN + NaCl.$$

The most common cyanohydrins are lactonitrile, $CH_3CH(OH)CN$; glycolonitrile, $HOCH_2CH$; and 2-methyl-lactonitrile, $(CH_3)_2C(OH)CN$. They are all water-white, highly reactive liquids which are very soluble in water, acetone, alcohol, and ether. The physical properties of lactonitrile are given in Table 2.6. Cyanohydrins are used chiefly as chemical intermediates in the production of pharmaceuticals and synthetic resin; under environmental conditions they can decompose to hydrogen cyanide or the cyanide ion. Cyanohydrins can be extremely toxic by ingestion, skin absorption, and eye contact (Fassett, 1963, p. 2020).

2.2.7 Cyanogenic Glycosides

Many plants are capable of releasing hydrogen cyanide (Conn, 1969). These plants do not contain hydrogen cyanide as such but rather contain cyanogenic glycosides, which have the general formula:



$$R_1 - \overset{\displaystyle CN}{\underset{\displaystyle R_2}{C}} - OR_3$$

where R_1 = an alkyl or aryl group,
R_2 = a hydrogen atom or methyl group,
R_3 = usually, D-glucose.

Amygdalin, the best-known representative of this group, is a β-glycoside of mandelonitrile (Table 2.6). It is found in the seeds and leaves of many members of the group Rosaceae (Williams, 1959, p. 402). Other important cyanogenic glycosides are prunasin, many stone fruits, other members of the Rosaceae group, sambunigrin (black elder leaves), dhurrin (sorghum), and linamarin and lotaustralin (white clover, lima beans, and cassava). Many common vegetables also contain cyanogenic glycosides: maize, millet, field bean, kidney bean, sweet potato, and lettuce (Oke, 1969).

In general, the pure glycoside is harmless; it becomes toxic only when in vivo conditions permit hydrolysis of the compound to liberate hydrogen cyanide. Formation of hydrogen cyanide frequently begins when the plant is crushed and proceeds rapidly to completion in the rumen or intestines of most animals (Oke, 1969). Further discussion of the cyanogenic glycosides is found in Section 4.2.3.

2.2.8 Complex Cyanide Compounds

Cyanide forms many complex compounds, especially with the transition metals, but only those few that are relevant to this discussion are described below.

2.2.8.1 Ferricyanides — Potassium ferricyanide, $K_3Fe(CN)_6$, is a ruby red, crystalline solid which is slowly soluble in water but only slightly soluble in alcohol. The aqueous solution is unstable and decomposes slowly on standing. Other physical properties are listed in Table 2.6. Potassium ferricyanide is widely used in making blueprints, staining wood, photography, dyeing wool, tempering iron and steel, electroplating, and in analytical chemistry (Stecher, 1968); it is frequently present in waste streams from such operations. The sodium salt, $Na_3Fe(CN)_6$, is less soluble in water but has properties similar to the potassium salt. Ferricyanides have relatively low toxicity because they do not normally liberate cyanide when acidified nor are they believed to be metabolized to cyanide in vivo (Arena, 1974, p. 211).

2.2.8.2 Ferrocyanides — Potassium ferrocyanide, $K_4Fe(CN)_6$, is a yellow efflorescent, crystalline solid which is soluble in water and insoluble in alcohol (Table 2.6). The aqueous solution decomposes slowly on standing. It is used in dyeing wool and silk, tempering steel, process engraving, photography, and in analytical chemistry. The sodium salt has similar properties and is used in blueprint paper, photography, pigments, dyes, and metallurgy (Stecher, 1968). Ferrocyanides are often present in waste streams from the various operations mentioned above. Like ferricyanides, the ferrocyanides have relatively low toxicity (Arena, 1974, p. 211).

Although neither ferrocyanides nor ferricyanides normally produce hydrogen cyanide or the cyanide ion, these toxic substances are readily formed when solutions of ferrocyanides or ferricyanides are treated with ultraviolet radiation, as from sunlight (Hendrickson and Daignault, 1973,

p. 6). The mechanism by which this result occurs was proposed by Lur'e and Panova (1964, cited in Hendrickson and Daignault, 1973):

$$4Fe(CN)_6^{4-} + O_2 + 2H_2O \rightarrow 4Fe(CN)_6^{3-} + 4OH^-,$$

$$4Fe(CN)_6^{3-} + 12H_2O \xrightarrow{h\nu} 4Fe(OH)_3 + 12HCN + 12CN^-.$$

These authors reported that in the presence of sunlight, 75% of the original ferrocyanide concentration in a solution was oxidized in five days and that the ferrocyanide disappeared completely in 10 to 12 days. This reaction has considerable toxicological significance. In an experiment performed by the U.S. Air Force Environmental Health Laboratory, Kelly Air Force Base, a photographic solution containing ferrocyanide killed no fish in 96 hr without sunlight present but generated over 220 times the LC_{50} of free cyanide in 6 hr when it was exposed to sunlight (Hendrickson and Daignault, 1973, p. 6). Clearly, ferrocyanide and ferricyanide wastes should not be discharged to streams where exposure to sunlight can occur.

2.2.8.3 Nitroferricyanides — Sodium nitroferricyanide, also known as sodium nitroprusside or sodium nitroprussiate, has the formula $Na_2(NO)Fe(CN)_5 \cdot 2H_2O$. It is a ruby red crystalline solid which is soluble in water but only slightly soluble in alcohol (Table 2.6). Aqueous solutions slowly decompose on standing. Sodium nitroferricyanide is used chiefly in analytical chemistry for the detection of organic compounds, SO_2, and alkali sulfides (Stecher, 1968). The potassium salt has similar properties.

Nitroferricyanides are used in the treatment of hypertensive disease (Page et al., 1955) and are believed to decompose in vivo, liberating cyanide (Section 6.2.2.1).

2.3 ANALYSIS FOR CYANIDES

2.3.1 Sampling and Sample Handling

Cyanides and cyanophoric substances may occur in the environment, and the principal requirements for handling each of these sample classes are discussed in the following sections.

2.3.1.1 Cyanides in Air — Hydrogen cyanide and other volatile cyanide-containing compounds are not normal constituents of air. They occasionally occur, however, as emissions from electrolytic plating plants, fumigation of buildings or ships with hydrogen cyanide, incomplete combustion of nitrogen-containing substances, or from chemical processing operations (Katz, 1968, p. 102). There is increasing concern with cyanide in the air due to its production by catalytic converters (as a pollution controlling device in automobiles) and by home fires due to the increased plastic content in homes. Samples are usually collected by drawing the polluted air through a liquid-filled bubbler or impinger-type collector. Countercurrent scrubbers and spray columns can also be used. These collectors are normally charged with an alkaline solution of sodium or potassium hydroxide. This solution is then processed as described in Section 2.3.1.2 prior to analysis. Hendrickson (1968) discussed sampling devices and procedures in detail.

2.3.1.2 Cyanides in Water — Cyanides do not normally occur in domestic wastewater; however, they are frequently important compounds of trade and industrial waste effluents (Leithe, 1973, p. 102). Cyanides may be present as free hydrocyanic acid, simple cyanide salts (e.g., sodium or potassium cyanide), varying decomposable cyanide complexes (e.g., potassium zinc salt), sparingly decomposable potassium ferricyanide, or one of various organic compounds (Section 2.2).

To minimize loss of the volatile hydrocyanic acid during storage, aqueous samples should be adjusted to pH 11 or greater by adding sodium hydroxide. After this pH adjustment, aqueous samples are frequently treated with a small quantity of lead carbonate and filtered to remove hydrogen sulfide which may interfere with the subsequent analysis. Volatile fatty acids, if present, are removed by acidifying the sample with acetic acid from pH 6 to 7 and extracting the aqueous phase with a small quantity of isooctane, hexane, or chloroform. Multiple extractions and long contact time should be avoided to minimize the loss of hydrogen cyanide (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971, p. 400). If oxidative impurities, such as free chlorine, are present, sufficient ascorbic acid is added to the sample to reduce them. The cyanide can be separated from any remaining impurities by treating the sample with sulfuric acid and distilling the resulting hydrogen cyanide into a receiver containing sodium hydroxide (Leithe, 1973, p. 104). Additives such as ethylenediaminetetraacetic acid, potassium iodide, or salts of copper(I), mercury, or magnesium may be used during the distillation to liberate cyanide. Analysis of the purified sample can now be performed by the analytical method of choice.

2.3.1.3 Cyanides in Biological Media — Cyanides may be recovered, with varying degrees of success, from most biologic materials by procedures similar to that described in Section 2.3.1.2; typically, the fluid or macerated tissue is warmed with water, lead, and trichloroacetic acid. The last reagent precipitates proteins and acidifies the sample, releasing hydrogen cyanide. The hydrogen cyanide is collected in a sodium hydroxide solution for subsequent analysis (Shanahan, 1973).

2.3.2 Methods of Analysis

Cyanide in environmental samples can be determined by a variety of procedures. Procedures which are currently important or which show promise of future usefulness are described in this section. The performance and limitations of each method are emphasized rather than minute operational details and are summarized in Table 2.8. Because of sensitivity, precision and accuracy obviously vary not only among different methods but also among various models of equipment and different operators (Karasek, 1975). Therefore, the tabulated data should be considered representative rather than definitive. Performance data cited by developmental laboratories usually are obtained under optimized conditions and procedures; interlaboratory comparisons, when they exist, offer more realistic comparisons of these characteristics.

TABLE 2.8. METHODS FOR DETERMINING CYANIDE

Analytical method	Important application	Limit of detection	Precision (relative standard deviation)	Accuracy (relative error)	Interfering substances	Selectivity
Absorption spectrophotometry	Natural and treated waters, trade and industrial effluents, biologic materials	0.5 μg in a 15-ml solution[a] 1 to 5 μg/liter[b] 0.02 mg/liter[c]	8.3% (0.06 mg/liter)[c] 15.1% (0.62 mg/liter)[c] 1.2% (40 μg/liter)[b]	2% to 7% (1.5 μg/liter)[b] 2% to 15% (0.28 to 0.62 mg/liter)[c]	Sulfides, thiocyanates, and fatty acids interfere but are removed by the sample preparation procedure.	Substances yielding cyanide when digested with sulfuric acid will be determined.
Volumetric titrimetry	Natural and treated waters and trade and industrial effluents where concentrations exceed 1 mg/liter cyanide	0.1 mg/liter[d]	2% (>1 mg/liter cyanide)		These are believed to be removed in the sample preparation step.	All cyanide-yielding substances will be determined.
Ion-selective electrodes	Natural and treated waters, industrial wastewaters	25 μg/liter[d]	0% to 5% (0.2 ppm cyanide)[d]	0% to 5% (0.2 ppm cyanide)[d]	Strongly complexing cations, sulfide	All substances which yield cyanide ions will be determined.
Indirect atomic absorption spectrophotometry	Industrial effluents polluted wastewater	60 ppb[e] (iron complex) 30 ppb[e] (silver cyanide)	2.2% (3 ppm)[e] 1.5% (2 ppm)[e]		None reported	Both techniques have good specificity for cyanide.
Fluorometry	Natural and treated waters, processed industrial effluents, biological materials	1 ppb[f]	11% (2.6 ppb)[f]		Sulfide, persulfate, ferricyanide, mercury (II), and iron(II) interfere.	Only cyanide ion is determined.
Gas chromatography	Natural and treated waters, industrial effluents, biologic fluids and solids	0.2 μg/ml[g] 25 ng/ml[h]	2.5% (10 μg/ml)[h]	2% (7 μg/ml)[g]	When treated with chloramine-T, thiocyanate yields a peak coincidental with cyanogen chloride which is about 2% or 3% of that from an equal concentration of cyanide.	Cyanide is determined.

Sources:

[a] American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971.
[b] Goulden, Afghan, and Brooksbank, 1972
[c] U.S. Environmental Protection Agency, 1974.
[d] Frant, Ross, and Riseman, 1972.
[e] Danchik and Boltz, 1970.
[f] Ryan and Holzbecher, 1971.
[g] Sass et al., 1971.
[h] Valentour, Aggarwal, and Sunshine, 1974.

2.3.2.1 Absorption Spectrophotometry — This analytical method is based on the formation of a colored molecular species of cyanide. The amount of absorbed light is compared with a previously determined calibration plot and is related to the cyanide concentration in the sample by the calibration data. Various absorption compounds are used by different analysts (Humphrey and Hinze, 1971; Leithe, 1973, p. 107; Nomura, 1968; Scoggins, 1972). Probably, the most frequently used compound is the blue dye formed by treating cyanide first with chloramine-T, then with an aqueous pyridine solution of bispyrazolone and 3-methyl-1-phenyl-5-pyrazolone. The detection limit, effective range, and relative standard deviation of this method are 0.5 μg in a 15-ml aqueous solution, 1 to 5 μg in a 25-ml aqueous solution, and approximately 2%, respectively. If the blue dye is concentrated by extraction into a small volume of organic solvent, greater sensitivity is obtained. When 10 ml of *n*-butyl alcohol is used, the sensitivity, effective range, and relative standard deviation is 0.1 μg, 0.2 to 2 μg, and 3.9%, respectively (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971, p. 406). A modification of the pyridine-pyrazolone method by Goulden, Afghan, and Brooksbank (1972) has a detection limit of 5 μg/liter cyanide. Sulfides, heavy metal ions, fatty acids, substances that hydrolyze to give cyanide ions, and oxidizing agents which are likely to destroy cyanide during the distillation step interfere with the determination of cyanide by this method, but they can be eliminated or minimized by the treatments described in Section 2.3.1.2.

Absorption spectrophotometric methods are widely used to determine cyanide in a variety of environmental samples, including natural and treated waters, trade and industrial effluents, and biological materials (Goulden, Afghan, and Brooksbank, 1972; Leithe, 1973, p. 107; Shanahan, 1973).

2.3.2.2 Volumetric Titrimetry — If the cyanide content of the alkaline distillate described in Section 2.3.1.2 is sufficiently large — 1 mg/liter or more (U.S. Environmental Protection Agency, 1974, p. 40), it can be determined by volumetric titration with an appropriate reagent. For example, silver nitrate combines with cyanide according to the equation:

$$2CN^- + AgNO_3 = [Ag(CN)_2]^- + NO_3^-.$$

One milliliter of 0.01 *N* silver nitrate is thus equivalent to 0.52 mg of cyanide ion. The indicator frequently used for this reaction is *p*-dimethyl-aminobenzalrhodanine. When the end point of the titration is reached, excess silver ions react with the indicator to produce a characteristic color change. A blank reagent value is subtracted from the result (Leithe, 1973, p. 106).

Nickel salts are also suitable titrants:

$$4CN^- + Ni^{2+} = Ni(CN)_4^{2-}.$$

One milliliter of 0.01 *M* nickel sulfate corresponds to 1.04 mg cyanide. Murexide, which is usually used as the indicator, changes from blue violet to orange yellow at the end point of the reaction.

Para-dimethylaminobenzalrhodanine, the indicator commonly used for the silver nitrate titration, is sensitive to about 0.1 mg/liter silver or to about 0.05 mg cyanide. The minimum detectable concentration of cyanide by the titration technique thus approaches 0.1 mg/liter if a 500-ml sample is used. The relative standard deviation is about 2% for distilled samples that contain at least 1 mg/liter cyanide (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971, p. 403). Volumetric titrimetry is commonly used as a supplement to the molecular absorption spectrophotometric method for analysis of cyanide in drinking, surface, and saline waters and in domestic and industrial wastes when the concentrations in these samples exceed 1 mg/liter cyanide.

2.3.2.3 Ion-selective Electrodes — Cyanide can be determined potentiometrically in certain industrial wastewaters by a specially designed electrode which contains a membrane of silver sulfide and silver iodide. When the electrode is immersed in a sample, iodide is released at the membrane surface in an amount proportional to the cyanide in the sample:

$$2CN^- + AgI \rightarrow Ag(CN)_2^- + I^-.$$

The liberated iodide is sensed by the electrode and determines the electrode potential. Consequently, the electrode response follows the relationship,

$$E = E_x + S \log [CN^-],$$

where $[CN^-]$ = the concentration of the cyanide ion,
S = slope of the electrode response curve.

Only free cyanide ions are detected; molecular hydrogen cyanide and other forms of complexed cyanide do not activate the electrode. Accordingly, samples must be adjusted to pH >11 before measurement to ensure the presence of cyanide as cyanide ion. In addition, strong complexing cations, such as nickel or copper, must be sequestered with ethylenediaminetetraacetic acid to free complexed cyanide prior to measurement. Sample color and turbidity do not interfere with the use of the cyanide-specific electrode and time-consuming sample distillations are eliminated. This method is therefore convenient for direct measurements in the field as well as for automated use in the laboratory. Although the silver sulfide-silver iodide membrane electrode can detect levels of cyanide as low as 50 ppb (Riseman, 1972), practical applications are currently limited to about 0.3 ppm (Frant, Ross, and Riseman, 1972).

Lower concentrations of cyanide can be measured by the electrode-indicator technique. In this method, a small volume of indicator solution, $Ag(CN)_2^-$, is added to a sample in which a silver sulfide membrane electrode is immersed. Three to five successive additions of a standard cyanide solution are then made to the sample, with observations of the electrode potential before and after each addition. A plot of these values on Gran's plot paper results in a straight line which extrapolates to the original cyanide concentration of the sample. The practical detection limit of the method is about 25 ppb. An upper limit, imposed by the formation of higher silver cyano complexes, is about 260 ppm (Frant, Ross, and Riseman, 1972). Metal ions which form stable cyanide complexes interfere but may be controlled by the addition of appropriate amounts of disodium ethylenediaminetetraacetate. Sulfide interferes and should be removed by the addition of a slight excess of lead(II). Other anions normally present in wastewater do not interfere.

The accuracy of the electrode-indicator technique is excellent; the relative error for synthetic samples containing 200 to 2000 ppb cyanide in the presence of several complexing cations was 0% to 5% (Frant, Ross, and Riseman, 1972). The method appears attractive for the analysis of natural waters and industrial wastes (Blaedel et al., 1971). Ion-selective electrode techniques have been discussed at length by Durst (1969).

2.3.2.4 Indirect Atomic Absorption Spectrometry — Cyanide cannot be determined directly by atomic absorption spectrometry; however, there are two general indirect techniques which can be used. In one technique, an insoluble metal cyanide compound is formed, and the metal in the precipitate or the excess metal in the supernatant solution is determined by atomic absorption spectrometry (Danchik and Boltz, 1970). The cyanide concentration is then computed from the measured concentration of the metal and the stoichiometry of the precipitation reaction. In the other general indirect technique, a stable metal-cyanide complex is formed, the complex is extracted, and the metal content of the extract is determined by atomic absorption spectrometry (Danchik and Boltz, 1970; Jungreis, 1969; Manahan and Kunkel, 1973). As before, the concentration of cyanide is computed from the measured concentration of the metal and the stoichiometry of the reaction.

Danchik and Boltz (1970) used both techniques in developing procedures for determining cyanide. In one instance, they converted the cyanide initially present in the sample to the dicyano-bis(1,10-phenanthroline)-iron(II) complex which was extracted in chloroform, dissolved in ethanol, and aspirated into the air-acetylene flame of an atomic absorption spectrophotometer. The absorption of iron is measured at 248.3 nm. The sensitivity of the method is about 60 ppb cyanide and the calibration is linear up to 5 ppm when ethanol is the solvent. The precision of the method is good; samples containing 2.85 ppm cyanide are determined with a relative standard deviation of 2.2%.

An alternative method of Danchik and Boltz (1970) is based on the precipitation of silver cyanide and the determination of the excess silver in the supernatant solution. The absorbance is measured in a luminous acetylene-air flame at 32.81 nm. The sensitivity of the method is good — about 30 ppb cyanide. The useful range is from 0.3 to 2.5 ppm cyanide. Samples containing 1.4 ppm cyanide are analyzed with a relative standard deviation of 1.5%.

Indirect atomic absorption spectrophotometric methods have sufficient sensitivity and precision for use in determining cyanide in many industrial effluents and other polluted wastewaters. However, they are not widely used, probably because of the general convenience and adequacy of competing methods.

2.3.2.5 Fluorometry — Fluorescein fluoresces when exposed to ultraviolet light, but its reduced form, the leucobase of fluorescein, is inactive until oxidized. Oxidation of the latter occurs readily in the presence of cupric and cyanide ions but not with cupric ions alone. These relationships form the basis of a very sensitive method for determining cyanide at the parts per billion level (Ryan and Holzbecher, 1971).

The analytical procedure consists of adding copper chloride, the leuco-base, and borate buffer to the sample to be analyzed. After the sample solution is mixed, it is irradiated with light of 488-nm wavelength; the resulting fluoresence is measured at 514 nm with a spectrofluorometer using 1-cm quartz cells. The intensity of the emitted light is related to the cyanide concentration of the sample by a previously prepared calibration curve. The detection limit of the method is excellent — less than 1 ppb cyanide; the relative standard deviation for samples containing 2.6 ppb cyanide is about 11%. Bromide, chloride, chlorate, fluoride, iodide, nitrate, acetate, thiocyanate, and sulfate ions do not interfere when present in concentrations up to 500 times the cyanide concentration. However, sulfide does interfere and must be removed. Persulfate and ferricyanide interfere strongly at ion to cyanide molar ratios of 1:1. Cobalt(II), nickel(II), and cadmium do not interfere in 500-fold excess. A 50-fold excess of zinc or aluminum, 25-fold excess of manganese, and a 5-fold excess of iron(III) also are without interference. Equivalent amounts of mercury(II) and iron(II) interfere and must be removed (Ryan and Holzbecher, 1971).

This laboratory-tested procedure has not received extensive testing under field conditions. However, it appears attractive for determining low concentrations of cyanide in natural and treated waters, processed industrial effluents, and biological materials. Other fluorometric methods for determining cyanide have also been published (Guilbault and Kramer, 1965; Guilbault, Kramer, and Hackley, 1967; McKinney, Lau, and Lott, 1972). Disadvantages of the fluorometric method reported to Guilbault are the lack of sensitivity and the lack of linearity in the response.

2.3.2.6 Gas Chromatography — Unlike analytical methods previously described, the gas chromatographic technique readily distinguishes various species of cyanide compounds. In general, hydrogen cyanide and other cyanide-containing compounds have different characteristic retention times in the chromatographic column and can be identified by this criterion. For example, Lo and Hill (1972) used this method to determine cyano compounds and goitrin in rapeseed meal. Sass et al. (1971) measured mixtures of various nitrites used by military and law enforcement agencies. Claeys and Freund (1968) used this technique to determine undissociated hydrogen cyanide in an aqueous cyanide solution. In their procedure, molecular hydrogen cyanide was removed from the sample by air sparging, concentrated in a cold trap, and analyzed in two serially connected columns containing 15% 1,2,3-tris(2-cyanoethyoxy)-propane on 60- to 80-mesh Chromosorb W/DMCS and 50- to 60-mesh Porapak Q, respectively. The carrier gas was nitrogen; a flame ionization detector was used. Samples containing 1 μg/liter hydrogen cyanide are readily analyzed. The calibration curve is linear up to 2000 μg/liter.

Valentour, Aggarwal, and Sunshine (1974) separated cyanide from blood, urine, gastric contents, and aqueous solutions by microdiffusion, treated the separated cyanide with chloramine-T, and extracted the resulting cyanogen chloride with hexane. The latter is measured with a 1.8-m (6-ft) long, 0.635-cm (1/4-in.) diameter, stainless steel chromatographic column packed with 7% Halcomid M-18 on 90- to 110-mesh Anakrom ABS and equipped with an electron capture detector. The sensitivity and precision of the method are good; as little as 0.25 μg/ml can be distinguished from the impurities in the blank and the relative standard deviation for samples containing 10 μg/ml cyanide is 2.5%.

The gas chromatographic technique is not widely used for determining cyanide in environmental samples because other methods are more convenient and less time consuming for routine samples. However, the inherent sensitivity and selectivity of the method ensure its application to specialized samples, especially those requiring differentiation of cyanide species.

2.3.3 Comparison of Analytical Procedures

At the present time, absorption spectrometry is probably the most widely used technique for determining cyanide in concentrations of 1 mg/liter or less (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971). Of the many variations of this technique, the Konig reaction, which involves the pyridine-pyrazolone reagent, is the most extensively used (Boltz, 1973, p. 218). This well-seasoned method is sensitive to about 0.5 μg cyanide in a 15-ml sample in its usual form and to 5 μg/liter with recent modifications (Goulden, Afghan, and Brooksbank, 1972). Its accuracy is adequate for analysis of cyanide in natural waters as well as treated industrial effluents. It is also amenable to automation.

Volumetric titration methods are "standard" and widely used in analyzing water samples and industrial effluents when the cyanide concentration is greater than 1 mg/liter (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971). The need for simple equipment and conventional laboratory procedure make this approach attractive in small laboratories.

The relatively new ion-selective electrode determination of cyanide is less versatile than the previously mentioned techniques, but it is attractive for selected application because sample preparation is usually eliminated and analyses are obtained speedily. The silver iodide membrane electrode is useful in the 10^{-3} to 10^{-5} M concentration range; above 10^{-3} M cyanide, the electrode life is shortened by the formation of soluble silver cyanide complexes (Riseman, 1972). Greater sensitivity can be achieved with the silver sulfide membrane electrode and the use of $KAg(CN)_2$ electrode indicator solution (Frant, Ross, and Riseman, 1972). Both techniques are amenable to automation, but strongly complexing cations must be controlled by adequate sample pretreatment.

Indirect atomic absorption spectrophotometric determinations of cyanide are based on well-established atomic absorption techniques for measuring such cations as iron(II) and silver (Danchik and Boltz, 1970). The sensitivity and precision of these methods are adequate for many treated industrial effluents and other polluted wastewaters. However, indirect atomic absorption methods are not yet widely used — probably because of the general convenience and adequacy of the absorption spectrophotometry and titrimetric techniques.

The determination of cyanide by fluorometry is relatively new and presently limited in application. Fluorometry is very sensitive — generally two orders of magnitude more sensitive than colorimetric procedures; analyses at or below 10 ppb are feasible, even in the presence of foreign ions (McKinney, Lau, and Lott, 1972). Accuracy at this concentration level is good (Ryan and Holzbecher, 1971). Few ions interfere, but mercury and sulfide are exceptions and must be removed. Nevertheless, fluorometry appears attractive for the analysis of cyanide in most natural and treated waters, as well as processed industrial effluents and wastewater.

The gas chromatographic method of determining cyanide is also sensitive and precise, particularly when it is converted to cyanogen chloride; in addition, it readily distinguishes speciated forms of cyanide-containing molecules. These characteristics commend its potential use for the analysis of cyanide from biological materials, gaseous and liquid effluents, and wastewaters, but it is little used at present.

SECTION 2

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SECTION 3

BIOLOGICAL ASPECTS IN MICROORGANISMS

3.1 SUMMARY

Microorganisms play an important role in the environmental conversion and cycling of many organic and inorganic compounds; they also serve as models for studying other biological systems. Although the details of the fate of cyanide compounds in the environment are not completely elucidated, microorganisms can degrade cyanide and, thus, may play an important role in detoxification of this substance.

Several species of bacteria and fungi can synthesize cyanide. An even wider variety of bacteria and fungi can degrade cyanide compounds introduced into the environment by industries or by cyanogenic plants. No data were found on the metabolism of cyanide by protozoa or yeast, except in conjunction with mixed populations. The production of cyanide by microorganisms has been associated with active growth and/or with autolysis. The importance of microbially produced cyanide in the ecosystem cannot be assessed at the present time. Unacclimated microbial populations may be very sensitive to cyanide (0.3 ppm cyanide is toxic to bacteria in sludge); however, acclimated populations in activated sludge can often completely oxidize nitriles to ammonia. Degradation of concentrations as high as 60 ppm CN^- has been reported.

The toxic effects of cyanide include those effects which are observable in whole cells or in populations: (1) alteration of respiration, (2) morphological changes such as swelling or filamentation, (3) increased lag periods for growth, (4) reduced biological oxygen demands, and (5) one case of induced mutation. Of the reported cyanide-induced effects on physiology, most studies are concerned with respiratory resistance and sensitivity. Inhibition of amino acid transport, alterations in nucleic acid metabolism, and inhibition of nitrogen metabolism are biochemical effects resulting from cyanide intake. The effects of cyanide are quite diverse among different microbial species. For further discussion of the effects of cyanide on microorganisms, the reader should consult Knowles' (1976) review.

3.2 METABOLISM

3.2.1 Uptake

Uptake of cyanide has been observed in several species of bacteria, fungi, and algae. No data were available on uptake of cyanide by protozoa or yeast. Evidence for uptake usually consists of demonstrating that cyanide can be metabolized by the microorganism. The carbon and nitrogen atoms of cyanide are transferred into a variety of metabolic end-products, depending on the species (Section 3.2.2.2). Whether cyanide is altered or converted before uptake into the cell is unknown. For example, Skowronski and Strobel (1969) reported that a strain of *Bacillus* *pumilus*, isolated from Fargo clay, decreased the concentration of cyanide in Trypticase soy yeast broth culture containing 0.1 *M* KCN (Figure 3.1). Growth was not observed in this medium. Cyanide was found to be actively metabolized; $^{14}CO_2$ was produced by the bacteria in the presence of $K^{14}CN$. Disappearance of cyanide coincided with the growth of *Bacillus megaterium* in broth culture containing a much lower KCN concentration (1 m*M*) (Castric and Strobel, 1969). Growth in the control medium (minus KCN) appeared at 3 to 4 hr, whereas growth in the test medium was not apparent until 10 to 12 hr. In fungi and an alga, addition of [^{14}C]cyanide has led to the production of labeled organic compounds, again suggesting that cyanide uptake occurs (Section 3.2.2.2).

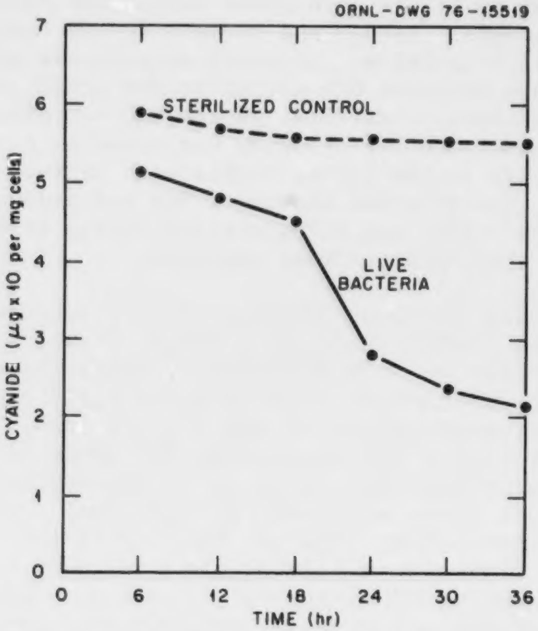

Figure 3.1. Amount of cyanide remaining in a medium containing 10^{-1} *M* KCN per milligram of a strain of *Bacillus pumilus* as a function of time. Source: Adapted from Skowronski and Strobel, Figure 2. Reproduced by permission of the National Research Council of Canada from the Canadian Journal of Microbiology, Vol. 15, pp. 93-98, 1969.

Knowles (1976) reviewed several other reports indicating microbial utilization of cyanide for growth. He suggested that cyanide might be assimilated by conversion to formic acid via formamide, after the action of cyanide hydratase and formamide hydrolyase. Another pathway could be similar to that described for cyanogenic organisms:

$$\text{glycine} \xrightarrow{C_1 \text{ unit}} \text{serine} \xrightarrow[\text{ATP(?)}]{\text{HCN}} \beta\text{-cyanoalanine} \longrightarrow \text{asparagine} \longrightarrow \text{aspartic acid} + NH_3 \ .$$

Other possible pathways of cyanide incorporation have been discussed by Howe (cited in Knowles, 1976).

3.2.2 Biotransformation

This discussion of biotransformation is concerned with the production and degradation of cyanide by microorganisms. The following reactions are discussed: carbon + nitrogen $\rightleftarrows$ cyanogenic compound $\rightleftarrows$ cyanide.

3.2.2.1 Production of Cyanide

3.2.2.1.1 Production by fungi — The basidiomycetes are the best-studied group of cyanogenic fungi. Lösecke (1871) was the first to describe a cyanogenic fungus — *Marasmius oreades*. Robbins, Rolnick, and Kavanagh (1950) detected HCN production by an unidentified fungus (isolated from white cedar) grown on liquid corn steep medium and associated the HCN production with autolysis. Lebeau and Dickson (1953) found that an unidentified psychrophilic basidiomycete which caused snow mold on alfalfa and other forage plants produced HCN during active growth on the mycelium as well as during autolysis. Mycelial growth was correlated with the quantity of HCN produced when the organism was grown on synthetic (Richard's solution plus pectin as the carbon source with various nitrogen sources) and plant media. The greatest amount of HCN was produced when the fungus was grown on soybean meal and alfalfa crown tissue (2500 and 700 ppm HCN, respectively, of the 2-g oven-dried samples).

Table 3.1 lists 31 known cyanide-producing species of fungi. *Pholiota aurea* has been studied in detail. Young, fresh, fruiting bodies of cultured *P. aurea* did not form HCN unless damaged (Bach, 1956). Older mycelia seemed to produce more HCN than young mycelia, and HCN production was greatest under relatively poor growth conditions. Dead cells of fruiting bodies and of mycelia did not exhibit HCN production. Thus, HCN production seemed to be dependent on labile enzyme systems and occurred only when the cells were grown under adverse conditions. Oxygen seemed to be required for HCN production. The optimum temperature for the reaction was about 20°C and the optimum pH for the basal solution was 5.9. Also, urea production increased as HCN increased. It was not determined whether urea was a precursor of HCN or was merely produced simultaneously. Bach found that *P. aurea* did not differ in its nutritional requirements from those of noncyanogenic fungi.

Ward and Lebeau (1962) correlated HCN production by an unidentified pathogenic basidiomycete (snow mold) with autolysis rather than with growth or with specific substrates. The particular isolate (type B) produced HCN in infected hosts (grasses and legumes) and in complex and synthetic media. Lebeau and Hawn (1963) found that the mycelial stage of *Marasmius oreades*, the fairy ring fungus, produced HCN when grown on malt-yeast-glucose medium and when found in lawns. Fruiting bodies also produced HCN. The production of cyanide may be important in the pathogenesis of *M. oreades* toward its grass host.

Ward (1964) reported that an unidentified sterile basidiomycete grown on malt extract, yeast extract, and glucose accumulated an unstable cyanogenic compound in the mycelium which yielded free HCN upon autolysis. Figure 3.2 shows that a bound form of HCN can be detected much earlier in the growth cycle than free HCN. Tapper and MacDonald (1974) subsequently established that the major cyanogen in snow mold extracts were glyoxylic acid cyanohydrin. Ward and Thorn (1965) have also extracted an unstable cyanogenic compound from *M. oreades*; its properties are similar to those of glyoxylic acid cyanohydrin.

TABLE 3.1. CYANOGENIC FUNGI

- *Mucoraceae*
  - *Mucor cyanogenes* Guyot
- *Polyporaceae*
  - *Polyporus frondosus* Dicks. ex Fries
  - *Polyporus giganteus* Pers. ex Fries
  - *Polyporus rutilans* Pers. ex Fries
  - *Trametes amygdalea* Maire
- *Agaricaceae*
  - *Leucospori*
    - *Cantharellus carbonarius* A. & S. ex Fries
    - *Clitocybe alexandri* (Gill.)
    - *Clitocybe clavipes* (Pers. ex Fries)
    - *Clitocybe cyathiformis* (Bull. ex Fries)
    - *Clitocybe geotropa* (Bull. ex Fries)
    - *Clitocybe gigantea* (Sow. ex Fries)
    - *Clitocybe infundibuliformis* (Schaeff. ex Fries)
    - *Clitocybe nebularis* (Batsch ex Fries)
    - *Clitocybe obbata* (Fries)
    - *Clitocybe parilis* (Fries)
    - *Marasmius alliaceus* (Jacq. ex Fries)
    - *Marasmius globularis* Fries
    - *Marasmius hariolorum* DC. ex Fries
    - *Marasmius oreades* Bolt. ex Fries
    - *Marasmius perforans* Hoffm. ex Fries
    - *Marasmius peronatus* Bolt. ex Fries
    - *Marasmius ramealis* Bull. ex Fries
    - *Marasmius rotula* L. ex Fries
    - *Collybia arborescens* Henn.
    - *Omphalia griseopallida* Desm. ex Fries
    - *Pleurotus porrigens* (Pers. ex Fries)
    - *Tricholoma cognatum* (Fries)
    - *Tricholoma goniospermum* Bres.
    - *Tricholoma nudum* (Bull. ex Fries)
  - *Ochrospori*
    - *Pholiota aurea* (Matt. ex Fries)
    - *Pholiota caperata* (Pers. ex Fries)

Source: Adapted from Bach, 1956, Table 14, p. 70. Data collected from several sources. Reprinted by permission of the publisher.

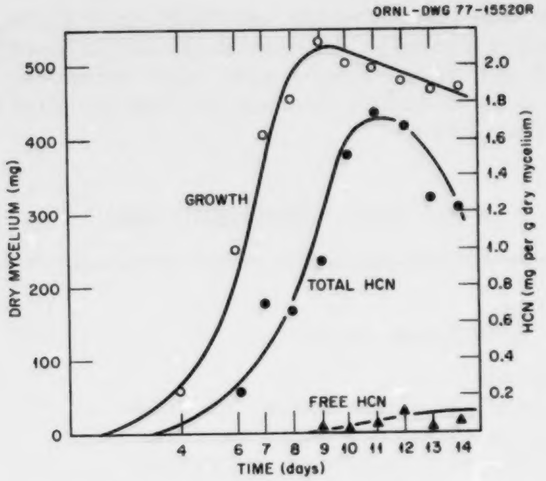

Figure 3.2. Production of HCN during growth of a snow mold fungus in shake culture. Free HCN collected by aeration, total HCN by steam distillation. Source: Adapted from Ward, Figure 1. Reproduced by permission of the National Research Council of Canada from the Canadian Journal of Botany, Vol. 42, pp. 319-327, 1964.

The mechanism of cyanide production in these organisms is not known. Stevens and Strobel (1968) reported that the snow mold converted valine and isoleucine to HCN via linamarin and lotaustralin (cyanogenic glycosides) (Section 4.2.3) as intermediates. However, Ward and Thorn (1966) and Ward, Thorn, and Starratt (1971) have clearly demonstrated that carbon-2 of glycine is the precursor of HCN in this organism. Ward, Thorn, and Starratt (1971) also provided strong evidence that the HCN supposedly produced from valine in Stevens' and Strobel's experiments was erroneously identified. Finally, Tapper and MacDonald (1974) were unable to detect linamarium or lotaustralin in the fungus as reported by Stevens and Strobel.

3.2.2.1.2 Production by bacteria — The production of cyanide by bacteria is well known. In early studies, Clawson and Young (1913) reported that several strains of *Bacillus pyocyaneus* (now classified as *Pseudomonas aeruginosa*) produced HCN when grown aerobically on gelatin, egg, broth, milk, agar, Dunham's peptone solution, and cotton-seed meal. *Bacillus violaceus* (*Chromobacterium lividum*) also produced HCN when grown on gelatin and egg. Patty (1921) found that oxygen was required for HCN production by *B. pyocyaneus* (*P. aeruginosa*); that HCN was not produced by a filtrable extracellular enzyme; that pigment production, gelatin liquefaction, and HCN production were independent but apparently related functions; that whole egg medium and a synthetic medium (as used in the methyl red test) supported the best HCN production; that pH optimum for HCN production was 5.4 to 5.8; and that the amount of HCN produced varied among strains. *P. aeruginosa* can produce cyanide when grown on a synthetic medium with glycine as the only nitrogen source (Lorck, 1948). After small amounts of nutrient broth or yeast extract were added to the synthetic glycine medium, HCN production increased. Hydrocyanic acid yield at 26°C was three times greater than at 37°C, and the maximum HCN concentration occurred at 25 hr and declined at 48 hr.

More recent work on cyanide production by *Pseudomonas* species has been done on a *Pseudomonas* isolate by Wissing (1968, 1974, 1975), on *P. aeruginosa* by Castric (1975), and on *P. fluorescens* by Freeman et al. (1975). A *Pseudomonas* strain isolated from a water reservoir produced the greatest amount of cyanide when grown on succinate plus glycine methyl ester or on glucose plus glycine and D,L-methionine (Wissing, 1968). The greatest amount of cyanide was produced just before the stationary phase, after which production rapidly decreased. If an additional carbon source was added to the cultures, HCN production again increased. The largest quantities of HCN were produced in Tris/HCl buffer at pH 8.3. From his studies, Wissing (1974) proposed that glycine ($H_2N{-}CH_2{-}COOH$) is oxidized in two steps, first to an imino acid ($HN{=}CH{-}COOH$) and then to HCN and CO_2. A linear relationship existed between the amount of glycine added to a culture and the amount of HCN produced (Figure 3.3). The strain used, designated as strain C, contained no *a* type cytochromes but contained *b*, *c*, and *o* types. Inhibitor studies suggested that the oxidative enzymes involved were flavoproteins. When *Pseudomonas* was treated with the oxidizing agent phenazine methosulfate before sonication, 8% of the HCN-producing activity of untreated intact cells was recovered in a cell-free preparation (Wissing, 1975). With the addition of flavinadenine dinucleotide (FAD), 16% of the activity was recovered. Gradient centrifugation and subsequent electron microscopy of the three fractions revealed that the main enzyme activity was associated with cytoplasmic membranes. The effect of FAD on HCN production indicates that flavoproteins may be involved in the bacterial conversion of glycine to HCN.

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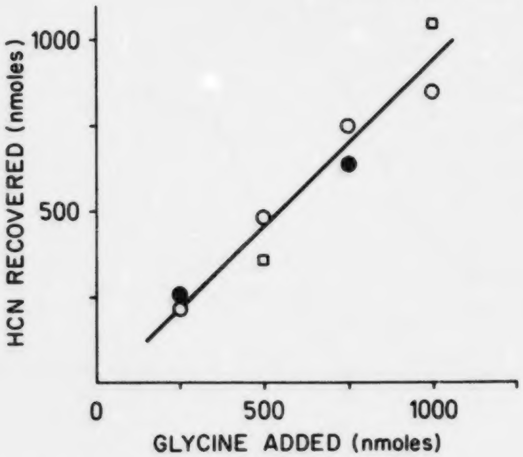

Figure 3.3. Regression line showing the molar ratio between added glycine and cyanide recovered when whole bacteria were fed varying amounts of glycine. Similar dots refer to experiments made with the same bacterial preparation. Source: Wissing, 1974, Figure 6, p. 1292. Reprinted by permission of the publisher.

Castric (1975) suggested that HCN is a secondary metabolite of *P. aeruginosa*. Strain 9-D2 (isolated from a septic human burn) grown in 2% peptone medium did not produce cyanide until the growth rate began to decline. Figure 3.4 shows three phases of growth and cyanogenesis: "(1) lag phase and active growth, no cyanide production, 0-6 h; (2) deceleration of growth, active cyanide production, 6-10 h; (3) limited growth and cyanogenesis, past 10 h." No further cyanide production was noted with incubation from 14 to 48 hr. The production of cyanide seemed to depend on protein synthesis, as shown by an 85% reduction in cyanide production with the addition of chloramphenicol (25 μg/ml, final concentration) to a 6-hr culture. Other factors affecting cyanogenesis were temperature, oxygen, and iron. Maximum cyanide production occurred at temperatures from 34°C to 37°C. Iron had a stimulatory effect at concentrations greater than 1 μ*M*. Anaerobic growth was inhibitory. Growth of strain 9-D2 in a synthetic medium lacking glycine decreased cyanide production by 80% (unpublished data by Castric, cited in Castric, 1975). This result is similar to that found with *Chromobacterium violaceum* (discussed below). The author suggested that cyanide may act as a regulator of glycine levels. Of the other species of *Pseudomonas* tested, only *P. fluorescens* and *P. polycolor* were cyanogenic. Several other bacteria (e.g., *Escherichia coli*, *Streptococcus faecalis*, and *Bacillus megaterium*) did not produce cyanide under test conditions.

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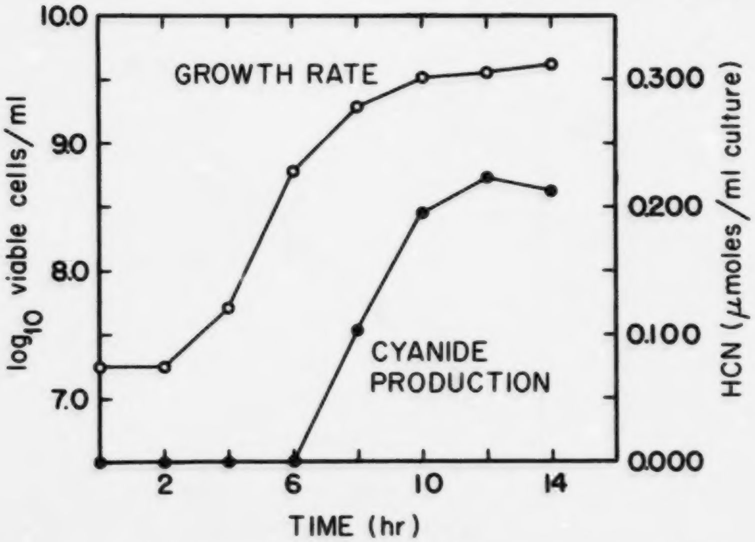

Figure 3.4. Time course of cyanide production in response to growth of *Pseudomonas aeruginosa* strain 9-D2 in 2% peptone at 37°C. Source: Adapted from Castric, Figure 1. Reproduced by permission of the National Research Council of Canada from the Canadian Journal of Microbiology, Vol. 21, pp. 613-618, 1975.

Freeman et al. (1975) used a very sensitive gas chromatography—mass spectrometry method to detect HCN production by *P. fluorescens*. They detected HCN production from two isolates grown on Trypticase soy agar plus 0.5% yeast extract or on sterile chicken medium. A closely related taxon, *P. putida*, did not exhibit cyanide biosynthesis, nor did *Flavobacterium, Cytophaga, Moraxella,* or *Acinetobacter* isolates.

Growing cultures and nonproliferating cells of *Chromobacterium violaceum* can synthesize HCN from the carbon-2 of glycine (Brysck, Corpe, and Hankes, 1969; Brysk, Lauinger, and Ressler, 1969; Michaels and Corpe, 1965; Michaels, Hankes, and Corpe, 1965). Cells grown in 1% (wt/vol) peptone medium produced the largest amount of HCN during the lag phase of growth (Figure 3.5) (Michaels and Corpe, 1965). A synergistic effect of glycine and methionine on cyanide formation by *C. violaceum* was found (Michaels and Corpe, 1965; Michaels, Hankes, and Corpe, 1965). The amount of cyanide produced depended on the concentrations of glycine and methionine in a glutamate-salt medium. A possible mechanism for the formation of CO_2 from the carboxyl group of glycine and of cyanide from the α-carbon is shown in Figure 3.6. However, neither the intermediates nor the enzymes proposed were demonstrated.

Thus, of the 34 bacterial species surveyed in this document, only seven species, representing two genera, were shown to be cyanogenic (Table 3.2): *P. aeruginosa, P. fluorescens, P. chlororaphis, P. aureofaciens, C. lividum,* and *C. violaceum.*

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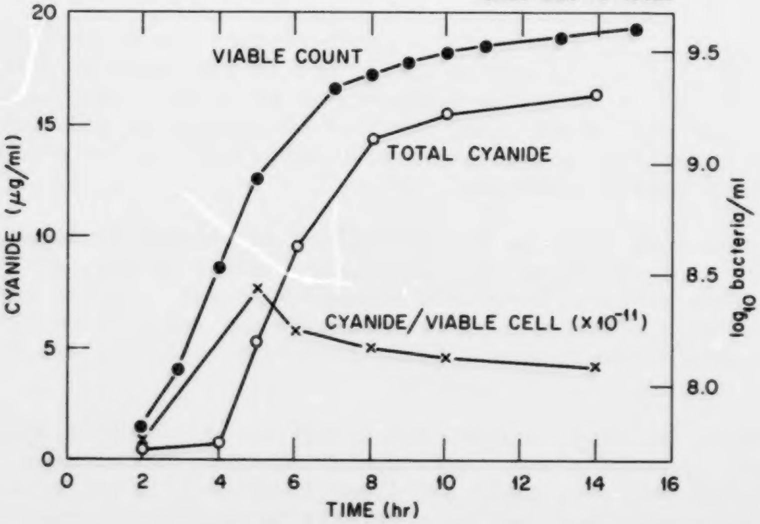


Figure 3.5. Time-course production of cyanide by *Chromobacterium violaceum* (in 1% peptone medium) in relation to growth. Source: Adapted from Michaels and Corpe, 1965, Figure 1, p. 109. Reprinted by permission of the publisher.

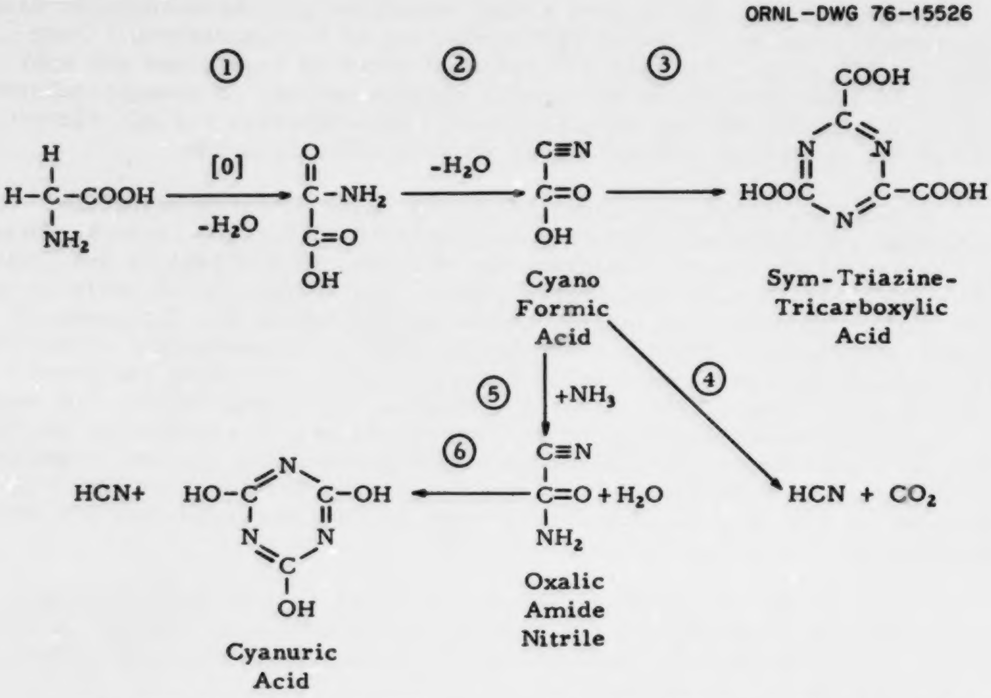


Figure 3.6. Hypothetical pathway for cyanide formation from glycine. Source: Michaels, Hankes, and Corpe, 1965, Figure 1, p. 124. Reprinted by permission of the publisher.

3.2.2.2 Degradation of Cyanide — Microorganisms are essential in the treatment of cyanide-containing wastes and in the natural purification of aquatic environments polluted with cyanide or related compounds. For example, Stolbunov (1971) found that the occurrence of thiocyanate-decomposing microorganisms seemed to be associated with points of industrial and household wastes discharge.

Few data were found on the metabolism of cyanide by algae. The green alga *Chlorella pyrenoidosa* can assimilate cyanide (Fowden and Bell, 1965). Thirty minutes after [^{14}C]cyanide was added to the culture, large amounts of radioactivity occurred in β-cyanoalanine and later (from 120 to 300 min) in γ-glutamyl-β-cyanoalanine. Cells grown anaerobically assimilated only negligible amounts of [^{14}C]cyanide.

Erickson, Maloney, and Gentile (1970) found that four species of phytoplankton, *Cyclotella nana*, *Amphidinium carteri*, *Skeletonema costatum*, and *Isochrysis galbana*, could not directly metabolize nitrilotriacetic acid, a chelating agent used as a builder in detergent formulations. Cyanide was not significantly toxic to these algae.

Data on the utilization of cyanide by protozoa and yeast were found only in conjunction with mixed populations.

TABLE 3.2. OCCURRENCE OF CYANOGENESIS IN BACTERIA

Organism	HCN production	Reference
Pseudomonas spp.		
P. aeruginosa	+	Clawson and Young, 1913
	+	Patty, 1921
	+	Lorck, 1948
	+	Castric, 1975
	-	Michaels and Corpe, 1965
P. alcaligenes	-	Castric, 1975
P. aureofaciens	+	Michaels and Corpe, 1965
P. cepacia	-	Castric, 1975
P. cichorii	-	Castric, 1975
P. chloroaphis	+	Michaels and Corpe, 1965
P. denitrificans	-	Castric, 1975
P. dimunata	-	Castric, 1975
P. fluorescens	+	Castric, 1975
	+	Freeman et al., 1975
	-	Michaels and Corpe, 1965
Pseudomonas isolate	+	Wissing, 1968, 1974, 1975
P. marginalis	-	Castric, 1975
P. maltophilia	-	Castric, 1975
P. polycolor	+	Castric, 1975
P. pseudoalcaligenes	-	Castric, 1975
P. putida	-	Castric, 1975
	-	Freeman et al., 1975
P. putrifaciens	-	Castric, 1975
P. stutzeri	-	Castric, 1975
Chromobacterium spp.		
C. lividum	+	Clawson and Young, 1913
	-	Michaels and Corpe, 1965
C. violaceum	+	Michaels and Corpe, 1965
	+	Michaels, Hankes, and Corpe, 1965
	+	Brysk, Corpe, and Hankes, 1969
	+	Brysk, Lauinger, and Ressler, 1969
	+	Ressler et al., 1973
Acinetobacter	-	Freeman et al., 1975
Alcaligenes oderans	-	Castric, 1975
Alcaligenes sp.	-	Castric, 1975
Bacillus megaterium	-	Castric, 1975
Bacillus subtilis	-	Michaels and Corpe, 1965
Bordetella bronchiseptica	-	Castric, 1975
Cytophaga	-	Freeman et al., 1975
Escherichia coli	-	Michaels and Corpe, 1965
	-	Castric, 1975
Flavobacterium sp.	-	Michaels and Corpe, 1965
	-	Castric, 1975
	-	Freeman et al., 1975
Herellea sp.	-	Castric, 1975
Mima sp.	-	Castric, 1975
Moraxella sp.	-	Castric, 1975
	-	Freeman et al., 1975
Serratia marcescens	-	Michaels and Corpe, 1965
Staphylococcus aureus	-	Michaels and Corpe, 1965
Streptococcus faecalis	-	Castric, 1975

3.2.2.2.1 Degradation by mixed populations — Mixed microbial populations, such as those found in activated sludge, may either be extremely sensitive to cyanide or may be able to degrade cyanide compounds in industrial wastes and in sewage. As little as 0.3 ppm cyanide can be toxic to bacteria in activated sludge (Berry, Osgood, and St. John, 1974). The disruption of a treatment process with cyanide may have more serious effects than the toxic effect of cyanide alone (Murphy and Nesbitt, 1964). All treatment processes are disturbed if shock loads of cyanide are added to unacclimated cultures. Unacclimated aerobic systems will produce poor quality effluents if more than 1 ppm cyanide is added (Corburn, 1949, and Lockett and Griffiths, 1948, both cited in Murphy and Nesbitt, 1964). Acclimation to cyanide apparently occurs when cultures are exposed to low cyanide concentrations. Rheinheimer (1974) stated that cyanides may kill organisms involved in the remineralization process in aquatic environments.

Several different additives can decrease the minimum retention time required for complete oxidation of thiocyanate and cyanide in a continuous activated sludge process for treating coke-oven wastes (Catchpole and Cooper, 1972). Alanine and *p*-aminobenzoic acid gave good results but proved uneconomical. Glucose was effective and economical. Pyruvic acid metabolism was thought to be an important factor in the improved treatment process. The acclimated complete-mixing activated sludge process (utilizing a feed of domestic sewage) was capable of degrading cyanide loads as high as 5 mg cyanide per gram mixed-liquor-volatile solids per hour with 99% efficiency (Murphy and Nesbitt, 1964). The rate of degradation was adequate for use with industrial effluents. Under equilibrium conditions of the system, one-third of the cyanide carbon was used by the sludge microorganisms for respiration and two-thirds was converted to cellular material. The occurrence of ammonia and nitrite in the effluent indicated some biological failure; the cyanide nitrogen is completely converted to nitrate in a properly functioning system. The above study was a pilot plant operation of a complete-mixing activated sludge process which proved to be a very successful, efficient, and economical means of degrading cyanide wastes. Some organisms isolated from a batch-fed system (600 ppm cyanide) which could rapidly decrease cyanide levels included: (1) a gram-positive coccus which grew slowly and destroyed cyanide in a few days, (2) a gram-positive sporulated bacterium which grew slightly faster, and (3) a mobile gram-negative *Pseudomonas* strain which grew rapidly (Rayand and Bizzini, 1959, cited in Murphy and Nesbitt, 1964).

Organic cyanides can also affect bacterial population structure and function. Ludzack et al. (1958), using Ohio River water from the intake of the Cincinnati Water Works, tested the effects of six different nitriles on the oxidation by and ecology of microbial populations. From qualitative observations (plate counts and stained slides made at irregular intervals), the variety of organisms was less in the nitrile-treated water than in the untreated river water, but often the total plate counts were higher in the treated water. Organisms found in systems treated with different organic nitriles are shown in Table 3.3. A greater variety of organisms was usually present in the lactonitrile- and oxydipropionitrile-fed systems. Specific organisms normally found in the river water were not given. Of the nitriles studied, oxydipropionitrile was the most resistant to microbial

TABLE 3.3. OCCURRENCE OF MICROORGANISMS IN NITRILE-FED OHIO RIVER WATER TEST OXIDATION SYSTEMS[a]

Nitrile	Bacteria		Molds		Protozoa	Yeasts
	Gram-negative cocci and bacilli	*Pseudomonas aeruginosa*	Several different hyphae and spores	*Trichoderma*		
Acrylonitrile	x		x			x
Acetonitrile	x				x	x
Adiponitrile	x				x	x
Benzonitrile	x				x	x
Lactonitrile	x	x	x	x		x
Oxydipropionitrile	x		x			x

[a] x indicates common occurrence.

Source: Adapted from Ludzack et al., 1958, p. 307.

oxidation, but after acclimation of the organisms it was rapidly consumed. Other authors have reported that acrylonitrile can be utilized by microorganisms. The Dow Chemical Company (cited in National Academy of Sciences, 1975) found that acrylonitrile could be 50% oxidized in ten days and completely oxidized in 20 days to NH_3 by an activated sludge seed. Ryckman, Rao, and Buzzell (1966) listed acrylonitrile among the compounds which could be removed from aquatic environments by acclimated microorganisms.

3.2.2.2.2 Degradation by fungi — The fungi *Fusarium solani* and *Stemphylium loti* may well be used to eliminate cyanide from industrial waste. *F. solani* can degrade cyanide to ammonia and CO_2 (Shimizu, Taguchi, and Teramoto, 1968; Shimizu and Taguchi, 1969; Shimizu et al., 1970). Shimizu, Taguchi, and Teramoto (1968) found that the overall cyanide degradation activity was 20 mg cyanide per milligram dry cell weight. The degradation rate decreased with increased age of the culture and with increased loading of the system. In further experimentation, the optimum cyanide concentration for maximum degradation was found to be less than 100 ppm cyanide (Shimizu, Fuketa, and Taguchi, 1969).

S. loti, a fungal pathogen of the cyanogenic plant bird's-foot trefoil (*Lotus corniculatus* L.), has a tolerance for HCN (Fry and Millar, 1972). Tolerant spores (adaptation affected by incubation in 0.1 m*M* KCN for 2 hr) or enzyme preparations from these spores convert hydrogen cyanide to formamide. The enzyme responsible for this conversion, formamide hydrolyase, has an optimum pH range of 7 to 9.

Pathways of cyanide metabolism have been proposed for the fungi which can utilize and detoxify cyanide. Table 3.4 lists the amounts of radioactivity incorporated into amino acids by various fungi (Allen and Strobel, 1966). *Rhizopus nigricans*, *Marasmius oreades*, and all species of *Pholiota* contained radioactive alanine. Other amino acids were also found to be radioactive, but to a lesser extent. *Fusarium nivale* contained labeled asparagine. Of the fungi tested, *Pholiota aurivella* appeared to assimilate the most HCN.

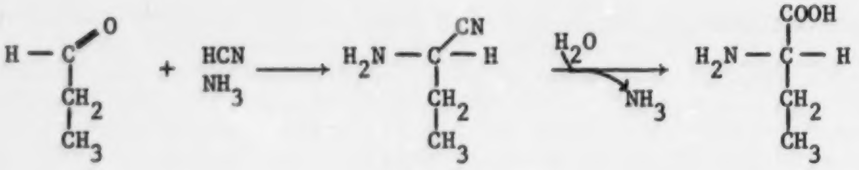
TABLE 3.4. COMPARATIVE ASSIMILATION OF $H^{14}CN$ BY VARIOUS FUNGI

Organism	Medium[a]	Days of growth	Dry wt (mg)	Radioactive amino acid detected	Specific activity (mμCi/μmole)
Pholiota adiposa	PD	34	42	Alanine	1.76
Pholiota aurivella	PD	28	36	Alanine	6.54
Pholiota praecox	PD	12	70	Alanine	5.86
Clitocybe illudens	PD	25	149	None	0.00
Marasmius oreades	PD	28	50	Alanine	4.14
Rhizoctonia solani	Syn	10	95	None	0.00
Fusarium nivale	Syn	14	90	Asparagine	0.43
Fusarium solani	Syn	7	130	None	0.00
Aspergillus flavus	Syn	8	93	None	0.00
Phoma betae	Syn	15	142	None	0.00
Rhizopus nigricans	PD	4	73	Alanine	1.58

[a] Syn — synthetic; PD — potato dextrose.

Source: Adapted from Allen and Strobel, 1966, Table 1. Reproduced by permission of the National Research Council of Canada from the Canadian Journal of Microbiology, Vol. 12, pp. 414-416.

Rhizoctonia solani can take up and metabolize HCN. Mundy, Liu, and Strobel (1973) proposed a pathway by which *R. solani* can condense $H^{14}CN$ with ammonia and propionaldehyde to form α-aminobutyronitrile:



propio-naldehyde **α-aminobutyronitrile** **α-aminobutyric acid**

They detected labeled α-aminobutyronitrile and α-aminobutyric acid after administering $K^{14}CN$ to drained cultures. The concentration of $K^{14}CN$ used was not stated but was said to be nontoxic. When labeled aminobutyric acic was administered to cultures, labeled CO_2 was detected in sufficient quantities to indicate its rapid metabolism. Precursor-product relationships suggested that α-aminobutyronitrile is probably a precursor of α-aminobutyric acid; enzymatic studies further substantiated this. An unidentified psychrophilic basidiomycete can combine KCN, succinic semialdehyde, and ammonia to form 4-amino-4-cyanobutyric acid, which can then be converted to glutamate (Strobel, 1967). Figure 3.7 illustrates how cyanide could be converted to CO_2 in a cyclical process.

3.2.2.2.3 Degradation by bacteria — Apparently, a wider variety of bacteria can degrade cyanide than can synthesize it. Such degradative organisms discussed in this section are *Corynebacterium*, *Arthrobacter*, *Bacillus*, *Thiobacillus*, *Escherichia coli*, and an *Actinomyces* isolate.

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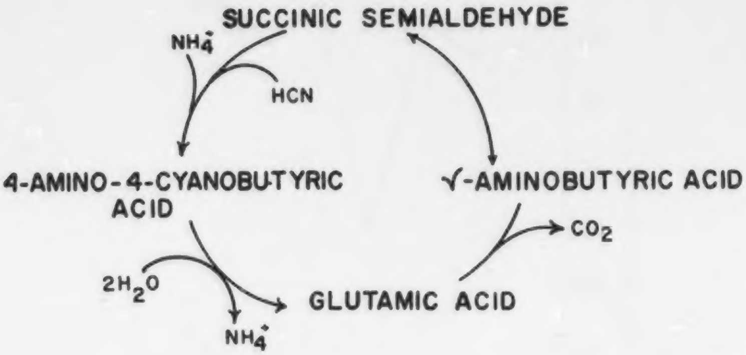

Figure 3.7. A cyclical process for converting HCN to CO_2 which involves glutamate, γ-aminobutyric acid, succinic semialdehyde, and 4-amino-4-cyanobutyric acid. Source: Adapted from Strobel, 1967, Figure 6, p. 3268. Reprinted by permission of the publisher.

Ware and Painter (1955) isolated a bacterium from sewage which could grow on silica gel medium with potassium cyanide as its sole source of carbon and nitrogen. The organism, which was "provisionally classed" among the Actinomycetaceae, had gram-positive branching filaments, was aerobic and autotropic, had aerial hyphae and conidia, and was inhibited in culture by agar or peptone. It utilized cyanide at concentrations up to 15 ppm, but the best growth occurred on cyanide concentrations of approximately 4 ppm. Ammonia was produced from the breakdown of cyanide, but the fate of the cyanide carbon was unknown.

An *Arthrobacter* strain was isolated by Aaslestad (1961, cited in Murphy and Nesbitt, 1964) from the same pilot plant used by Murphy and Nesbitt. Cultures of this strain could degrade concentrations of cyanide up to 60 ppm. Aaslestad hypothesized that cyanide could act as a sole nitrogen source but not as a sole carbon source for the organism. Ammonia inhibited a growing culture. The strain could also convert cyanide to thiocyanate in the presence of sulfur; however, thiocyanate was not thought to be an intermediate in the normal degradation process.

An unclassified cyanide-resistant bacterial strain from soil polluted with wastewater from an electroplating plant exhibited three peaks of cyanide-degrading activity at pH 5.3, 7.0, and 10.0 (Figure 3.8) (Furuki et al., 1972). At pH 10.0, cyanide degradation was highest in the exponential growth phase; the organism grew well in alkaline media containing 400 to 500 ppm cyanide.

The major pathway for cyanide assimilation in higher plants is the reaction with cysteine to form β-cyanoalanine (Section 4.2.1). A variant of this pathway involving serine instead of cysteine is apparently found

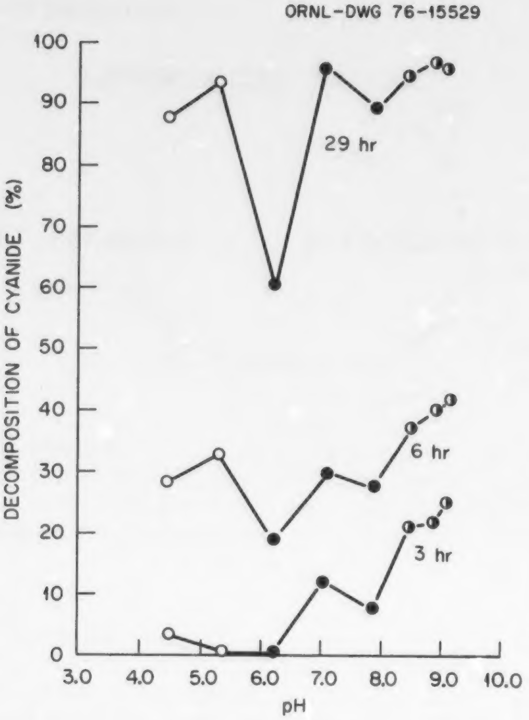

Figure 3.8. Effect of pH on the degradation of cyanide by a cyanide-resistant bacterium. Source: Adapted from Furuki et al., 1972, Figure 11, p. 302. Reprinted by permission of the publisher.

in some bacteria (Knowles, 1976). Brysk, Corpe, and Hankes (1969) and Brysk, Lauinger, and Ressler (1969) reported that nonproliferating *Chromobacterium violaceum* cells incubated with glycine, methionine, and succinate accumulated β-cyanoalanine (Figure 3.9); these workers postulated serine as the acceptor of HCN. Young cultures of *C. violaceum* also accumulated γ-cyano-α-L-aminobutyric acid (Brysk and Ressler, 1970). The formation of γ-cyano-α-L-aminobutyric acid by the organism was greatest at the end of the lag phase and decreased with increasing age. Ressler et al. (1973) have described the reactions involved and have purified the enzyme involved. The only β-cyanoalanine synthetic activity in cell-free extracts of *Bacillus megaterium* was associated with *O*-acetylserine sulfhydrase (Castric and Conn, 1971). This enzyme converts $Na^{14}CN$ and either *O*-acetyl-L-serine or L-cysteine to β-cyanoalanine-^{14}C. L-Serine and cyanide produced much less β-cyanoalanine. Since the authors detected no cysteine-linked β-cyanoalanine synthetase in extracts, they suggested that this enzyme either was lacking in this strain or was labile and easily inactivated.

Bacteria which are fairly resistant to cyanide are sometimes found in the environment of cyanogenic plants. A strain of *Bacillus pumilus* isolated from Fargo clay cropped in flax (a cyanogenic plant) for 73 years

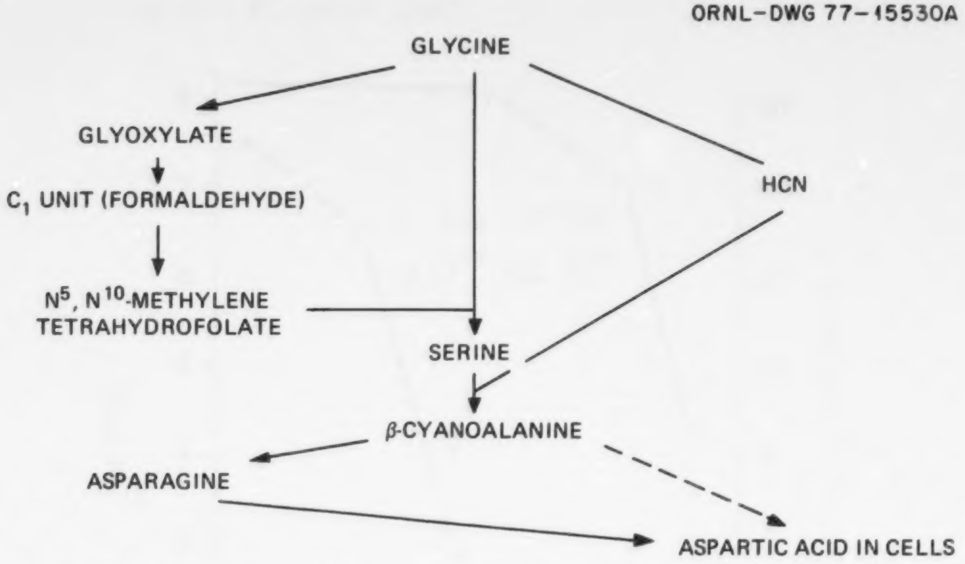

Figure 3.9. Proposed pathway for the incorporation of cyanide by *Chromobacterium violaceum*. Source: Adapted from Brysk, Corpe, and Hankes, 1969, Figure 2, p. 326. Reprinted by permission of the publisher.

could survive in saturated KCN solutions (Skowronski and Strobel, 1969). Labeled $^{14}CO_2$ and $^{15}NH_4^+$ were produced from the metabolism of $K^{14}C^{15}N$ by the organism. Bacteria obtained from the standard medium with 10^{-1} *M* KCN were incubated in a modified Dulbecco's medium with 10^{-1} *M* KCN and 11 μCi of $K^{14}CN$. In the nitrogen experiment, all conditions were the same except that 2 mg of $K^{15}CN$ instead of $K^{14}CN$ was introduced to the medium. Figure 3.10 shows that $^{14}CO_2$ production stopped at 36 hr and $^{15}NH_3$ was produced primarily from 36 to 54 hr, suggesting that the cyanide nitrogen was preferentially retained by the cells during the most active growth period.

The enzyme rhodanese, which catalyzes the formation of thiocyanate and sulfite from cyanide and thiosulfate, is found in human and animal tissues (Section 6.2.3.1) and has also been isolated from several different bacterial species: *Thiobacillus denitrificans* (Bowen, Butler, and Happold, 1965), *Escherichia coli* (Stearns, 1953, cited in Oke, 1969), *Thiobacillus thiocyanooxidans* (McChesney, 1957, cited in Oke, 1969), *Bacillus subtilus*, *Bacillus coagulans* (Villarejo and Westley, 1963), and *Bacillus stearothermophilus* (Atkinson, 1975; Atkinson, Evans, and Yeo, 1975; Villarejo and Westley, 1963). Villarejo and Westley (1963) detected no rhodanese activity from *E. coli* K12, *E. coli* W, and certain *Neurospora crassa* strains.

A high level of rhodanese has been found in *B. stearothermophilus* (Atkinson, 1975). When used in a small chemical reactor for the continuous removal of cyanide as thiocyanate, the organism could remove quantities as high as 5 to 8 g NaCN per liter of culture per hour at 27°C.

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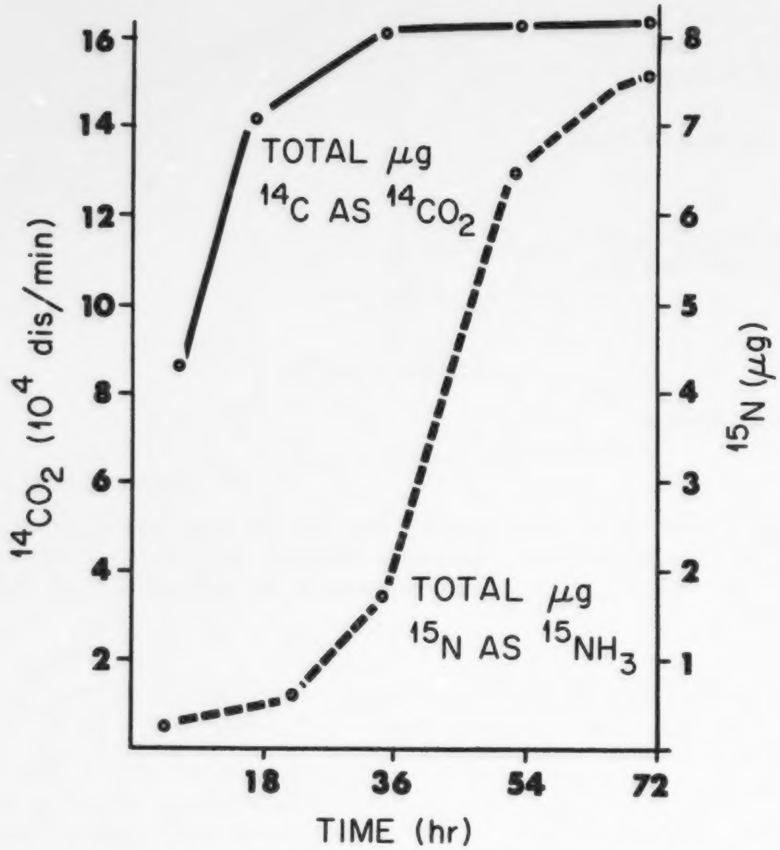

Figure 3.10. Production of $^{14}CO_2$ and $^{15}NH_3$ by a strain of *Bacillus pumilus* as a function of time in cells exposed to $K^{14}C^{15}N$. Source: Adapted from Skowronski and Strobel, Figure 3. Reproduced by permission of the National Research Council of Canada from the Canadian Journal of Microbiology, Vol. 15, pp. 93-98, 1969.

For rhodanese activity, the optimal ratio of thiosulfate to cyanide was 1.5-2.5:1 for intact cells and 1:1 for purified rhodanese in vitro. Atkinson has also developed a mutant strain of *B. stearothermophilus* which is resistant to 10^{-3} *M* NaCN and contains five to six times the rhodanese activity of normal cells. Atkinson, Evans, and Yeo (1975) found that rhodanese production seemed to be independent of medium composition and was higher in continuous culture than in batch cultures.

Corynebacterium nitrilophilus can assimilate organic nitriles such as acetonitrile, adiponitrile, butyronitrile, propionitrile, and succinonitrile (Mimura, Kawano, and Yamaga, 1970). The organism has been proposed as a useful addition to activated sludge for treatment of nitrile wastes.

3.3 EFFECTS

3.3.1 Growth Effects

The effects of cyanide on the morphology and growth of microorganisms include increased lag times for growth, altered cell morphology, decreased biological oxygen demand (BOD), and decreased motility. The alga *Scenedesmus quadricauda* was very sensitive to cyanide, having a toxicity threshold of 0.16 ppm cyanide, whereas 20 ppm was toxic to the alga *Microcystis aeruginosa* (Table 3.5). As previously stated (Section 3.2.2.2.1), sewage organisms can be quite sensitive to low cyanide concentrations (0.3 to 1 ppm cyanide). Fifty percent of the BOD of sewage organisms was inhibited by 15 ppm cyanide (Table 3.5).

3.3.1.1 Protozoa — Table 3.5 shows the effects of cyanide, ferrocyanide, and thiocyanate on a variety of microorganisms. The protozoan *Microregma heterostoma* was the most sensitive to cyanide with a toxicity threshold of 0.04 ppm CN^-.

Sodium cyanide is also lethal to several other protozoa. Willard and Kodras (1967) reported that 50 ppm sodium cyanide caused death or disintegration of all bovine rumen protozoa in 24 hr (protozoa with no internal or external cilia movement were considered dead). In the control sample, approximately 50% of the protozoa were dead at 24 hr. A diverse population of rumen protozoa was observed: oligotrichs found were *Entodinium*, *Ophryoscolex*, *Polyplastron*, *Metadinium*, and others; holotrichs found were *Isotrichia* and *Dasytricha*.

3.3.1.2 Bacteria — Few reports presenting cyanide toxicity data for bacteria were found. *Escherichia coli* had a toxicity threshold of 0.4 to 0.8 ppm cyanide (Table 3.5). Some bacteria can develop substantial respiration in the presence of cyanide (Section 3.3.2.1) (Henry and Nyns, 1975); however, effects on the growth of these cultures are not usually given. Examples of extreme resistance have been reported.

Two gram-negative rod-shaped bacteria, isolated from the rhizosphere of the cyanogenic cassava or tapioca plant (*Manihot utilissima*), could tolerate up to 50 ppm KCN in soil extract agar (Sadasivam, 1974). Potassium cyanide had little effect on the mean generation time of *E. coli* when D-xylose was used as the carbon source (Ashcroft and Haddock, 1975). The generation time was 1.5 hr without cyanide and 2.3 hr in the presence of 1 m*M* KCN. When sodium succinate was used as the carbon source, however, no growth was detected with concentrations greater than 0.25 m*M* KCN. With 0.15 m*M* KCN, the mean generation time increased to 7 hr from the control value of 2.4 hr.

Using phase-contract microscopy, Ingram, Thurston, and Van Baalen (1972) observed no morphological changes in cells of the blue-green bacterium *Agmenellum quadruplicatum* exposed to KCN (concentration not given). Chloramphenicol and penicillin G induced temporary filamentous forms. A strain of *Bacillus pumilus* grown in 0.1 *M* KCN did form long filaments after 60 min (Skowronski and Strobel, 1969). These filaments did not revert back to normal morphology when they were put back into medium with KCN. The organism could survive in concentrations of 2.5 *M* KCN.

TABLE 3.5. TOXICITY OF CYANURATES AND CYANIDES TO MICROORGANISMS

Chemical compound	Test organism	Test conditions[a]	Concentration (ppm)	Remarks
Ammonium thiocyanate	Sewage organisms	FW, LS	>5000	50% inhibition of BOD[b], 20 °C
Potassium cyanide	*Microcystis aeruginosa* (algae)	FW, LS	20	Lethal; lab study on algae culture (90% kill)
	Sewage organisms	FW, LS	15.0	50% inhibition of BOD, 20 °C
	Scenedesmus quadricauda (algae)	SB, FW, LS	0.16	Toxicity threshold, four days at 24°C
	Escherichia coli (bacteria)	SB, FW, LS	0.4–0.8	Toxicity threshold, one to two days at 27°C
	Microregma heterostoma (protozoa)	SB, FW, LS	0.04	Toxic threshold, 28 hr, 27 °C
Potassium ferrocyanide	*Scenedesmus quadricauda* (algae)	SB, FW, LS	0.15	Toxic threshold, four days at 24°C
	Escherichia coli (bacteria)	SB, FW, LS	1000	No adverse effect, 27 °C
	Synthetic sewage	FW, LS	0.75	50% reduction BOD

[a] SB – static bioassay; FW – freshwater; LS – lab study.
[b] BOD – biological oxygen demand.

Source: Adapted from Becker and Thatcher, 1973, Table J, pp. J.2–J.10. Data collected from several sources.

Bowdre and Krieg (1974) proposed that cessation of motility of the bacterium *Spirillum volutans* be used as an indicator of toxicants in industrial effluents. However, the only nitrile compound tested in this laboratory study, nitrilotriacetic acid (90 ppm, neutralized with KOH), did not affect the motility of the cells even after 75 min.

Blum, Nolte, and Robertson (1975) tested the effects of cyanoacrylate compounds on possible resident and/or pathogenic microorganisms of the body. These compounds have been used as tissue adhesive and hemostatic agents in medicine and dentistry and may possibly be used in peridontal surgery. *Pseudomonas aeruginosa* and the fungus *Candida albicans* were resistant to isobutyl and trifluoro cyanoacrylate compounds, as determined by disc sensitivity tests. *Staphylococcus aureus* was the most sensitive organism tested. *Lactobacillus casei* was slightly resistant. The authors suggested that the growth inhibition observed in their plating assays was due to vapors rather than diffusion of the cyanoacrylates.

3.3.1.3 Fungi — Similarly, few data are available on the effects of cyanide on the growth of morphology of fungi. An *Aspergillus* sp. and *Rhizopus nigricans* grown on potato dextrose agar tolerated 100 ppm and 200 ppm KCN respectively (Sadasivam, 1974). If such organisms could metabolize cyanide from the root region of cyanogenic plants, toxicity to other neighboring organisms might be nullified. Cyanide as an alleopathic agent has not been well documented, however.

A 50% reduction in growth of *Saccharomyces cerevisiae* with no effect on average cell size resulted from additions of *n*-butyronitrile (4000 ppm), 3-cyanopyridine (1000 ppm), propionitrile (4000 ppm), and *m*-valeronitrile (4000 ppm) (Loveless, Spoerl, and Weisman, 1954). Approximately 50% reduction in growth of *E. coli* cells with no effect on cell size resulted from additions of 1000 ppm acrylonitrile, 1000 ppm β-chloropropionitrile, and 3 ppm sodium cyanide (Loveless, Spoerl, and Weisman, 1954). Compounds tested by Loveless, Spoerl, and Weisman which inhibited both growth and division of *S. cerevisiae* are listed in Table 3.6.

3.3.2 Metabolic Effects

3.3.2.1 Respiration — Data on the metabolic effects of cyanide on microorganisms deal primarily either with respiratory sensitivity or with enhancement. Examples of both cyanide-insensitive and cyanide-sensitive respiratory chains are found in fungi, algae, protozoa, and bacteria (Henry and Nyns, 1975).

3.3.2.1.1 Protozoa — Effects of cyanide on protozoal respiration include enhancement, resistance, and sensitivity. Various protozoa exhibit a degree of cyanide-insensitive respiration at some stage during their life cycles (Henry and Nyns, 1975). Bloodstream forms are apparently insensitive to cyanide. They lack mitochondria, a functional Krebs cycle, and an electron transport chain. A pleomorphic strain of *Trypanosoma* lacked cytochromes and a fully active Krebs cycle, and its respiration was insensitive to cyanide (Hanas, Linden, and Stuart, 1975). Ray and Cross (1972) proposed a branched electron transport chain in *Trypanosoma mega* in which

TABLE 3.6. COMPOUNDS INHIBITING BOTH GROWTH AND DIVISION OF *SACCHAROMYCES CEREVISIAE*

Compound	Concentration in culture medium (ppm)	Percent of control weight	Percent of control size
Acrylonitrile	1000	52	170
Cyanamide	300	37	160
Cyanoacetic acid	4000	45	220
Malononitrile	40	62	150
Sodium cyanide	125	38	150

Source: Adapted from Loveless, Spoerl, and Weisman, 1954, Table 1, p. 640. Reprinted by permission of the publisher.

one branch contained cytochromes *c* and a-a_3 and was cyanide sensitive. The other branch contained a cyanide-insensitive oxidase identical to cytochrome *o*. The cyanide-sensitive oxidase was inhibited by low concentrations of carbon monoxide, and the cyanide-insensitive oxidase was inhibited only at high carbon monoxide concentrations.

Calvayrac and Butow (1971) found that increased respiration via a cyanide-resistant pathway occurred when antimycin A was added to *Euglena gracilis*, strain Z, growing on lactate. Giant mitochondria were observed several hours after the addition of antimycin. Electron micrographs showed altered structure with many cristae in the matrix. Succinic oxidase from *Crithidia fasciculata* mitochondria was inhibited 50% at concentrations of 5 μ*M* KCN and 99% at 50 μ*M* KCN (Kusel and Storey, 1973). The organism appeared to have cytochrome a_3 as its sole terminal oxidase. No cytochrome *o* was detected.

3.3.2.1.2 Bacteria — Bacterial respiratory systems are inhibited by low concentrations of cyanide (Slater, 1967). Gel'man, Lukoyanova, and Ostrovskii (1967) have listed the effects of cyanide on many bacterial respiratory enzymes (Table 3.7). Recent studies on cyanide-resistant respiratory chains deal with the following bacteria: *Azotobacter vinelandii* (Jones and Redfearn, 1967; Jones, 1973), *Beneckea natriegens* (Weston, Collins, and Knowles, 1974), *Escherichia coli* (Ashcroft and Haddock, 1975; Pudek and Bragg, 1974, 1975), *Photobacterium phosphoreum* (Yoshikawa and Oishi, 1971), *Chromatium* strain D (Takamiya and Nishimura, 1974), *Rhodopseudomonas capsulata* (Melandri, Zannoni, and Melandri, 1973; Zannoni et al., 1974), *Pseudomonas saccharophila* (Donawa, Ishaque, and Aleem, 1971), *Bacillus cereus* (McFeters, Wilson, and Strobel, 1970), and *Achromobacter* strain D (Arima and Oka, 1965).

TABLE 3.7. EFFECT OF CYANIDE ON BACTERIAL RESPIRATORY CHAINS

Bacterium	Concentration (*M*)	Enzyme system	Inhibition (%)
Pseudomonas sp.	10^{-3}	Malic oxidase	50-80
Pseudomonas aeruginosa	3×10^{-4}	Cytochrome c_{551}:nitrite:O_2 oxidoreductase	96
	10^{-4}	Cytochrome *c*:O_2 oxidoreductase	96
Aerobacter aerogenes	10^{-3}	Succinic oxidase	95
	10^{-3}	Pyruvic oxidase	95
	10^{-4}	Pyruvic oxidase	50
Azotobacter vinelandii	10^{-3}	NADH oxidase	85
	2×10^{-4}	NADH oxidase	48
	10^{-2}	Succinic oxidase	80
	10^{-3}	NADH:nitrate reductase	70-90
	10^{-3}	NADH oxidase	100
		Succinic oxidase	100
Pasteurella tularensis	6×10^{-2}	NADH oxidase	80
Staphylococcus aureus	10^{-3}	NADH oxidase	60
Mycobacterium phlei	3×10^{-3}	Succinic oxidase	95
		NADH oxidase	55
Acetobacter xylinum	10^{-3}	Malic oxidase	100
Sarcina lutea	10^{-3}	Succinic oxidase	96
Escherichia coli	7×10^{-3}	Succinic oxidase	100
		Malic oxidase	100
Corynebacterium diphtheriae $PW8_sP$	2.5×10^{-3}	Succinic oxidase	50-60
Myxococcus xanthus cells	10^{-3}	NADH oxidase	78
Myxococcus xanthus microcysts	10^{-3}	NADH oxidase	29
Micrococcus denitrificans	10^{-4}	Cytochrome *c* oxidase	100
Acetobacter peroxydans	10^{-3}	Lactic oxidase	90
Xanthomonas phaseoli	5×10^{-3}	Succinic oxidase	87
Proteus vulgaris	3.3×10^{-3}	NADH oxidase	84

Source: Adapted from Gel'man, Lukoyanova, and Ostrovskii, 1967, Table 19, p. 142. Data collected from several sources. Reprinted by permission of the publisher.

3.3.2.1.3 Fungi — Some of the fungal genera containing cyanide-resistant respiratory systems are *Moniliella*, *Candida*, *Neurospora*, and *Saccharomyces*. *Moniliella tomentosa* has a branched respiratory chain; one branch is cyanide sensitive and contains cytochromes *a*, *b*, and *c*, while the other branch is cyanide insensitive (Hanssens, D'Hondt, and Verachtert, 1974). A variant strain of *Candida utilis* is less copper dependent than the wild type because of a terminal oxidase which bypasses cytochrome oxidase and cytochrome *c*. This alternate pathway is insensitive to cyanide (Downie and Garland, 1973). A comparison of the respiratory chains of wild-type and *poky Neurospora crassa* strains was made by Lambowitz and Slayman (1971). Wild-type cells were sensitive to cyanide, having a cytochrome chain similar to that of higher organisms. However, *poky* cells possessed two alternative oxidase systems — one similar to that of the wild type and thus cyanide sensitive and the other insensitive to cyanide concentrations which maximally inhibit the cytochrome chain. Schwab (1973) reported that O_2 uptake of mitochondria from copper-depleted *N. crassa* cells was only slightly sensitive to 1 m*M* KCN (O_2 uptake was 94% of control mitochondria). The insensitivity was due to a branched respiratory chain; one branch contained a cyanide-insensitive oxidase perhaps similar to the oxidase system in *N. crassa* described by Lambowitz and Slayman (1971). Resting cells of *M. tomentosa* exhibited approximately 60% inhibition of O_2 uptake by 10^{-3} *M* cyanide, whereas more aged cells (48-hr-old culture) were even more resistant to cyanide — 30% inhibition with 10^{-3} *M* cyanide (Hanssens, D'Hondt, and Verachtert, 1974).

Bergquist et al. (1974) described the pleiotropic effect of mutations occurring at loci affecting isoleucine and valine biosynthesis in *N. crassa*. The cytochromes of the mitochondria from mutants were altered, as shown by reduced O_2 uptake, different cyanide sensitivities, different ratios of cytochrome *b* to *a*, and generally lower cytochrome concentrations. Oxygen uptake of the wild-type strain was inhibited by concentrations of KCN as low as 0.01 m*M*, but the wild type did contain a component which was relatively cyanide resistant. Three of the mutant strains tested were more resistant to cyanide than was the wild type.

Concentrations of 10^{-5} to 10^{-3} *M* KCN inhibited oxygen consumption by 11% to 25% in cultures of the fungus *Fusarium lini* (Weiss-Berg and Tamm, 1971). However, if the steroids deoxycorticosterone and digitoxigenin are added with KCN, oxygen consumption is increased above controls. Presumably, this increase in O_2 uptake is due to hydroxylation of the steroids, and the function of cyanide is to inhibit cytochrome oxidase.

Von Jagow and Klingenberg (1970) used ferricyanide as an electron acceptor for localizing two dehydrogenases in *Saccharomyces carlsbergensis*. Both enzymes were connected to the cytochrome chain via the ubiquinone pool. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and KCN were used as uncouplers in studying respiration of wild-type and petite strains of *S. cerevisiae*. Cyanide (3 m*M*) and CCCP (10^{-6} *M*) caused degradation of mitochondria in yeast (*S. cerevisiae*, wild type) cells.

3.3.2.1.4 Algae — The respiration of most algae apparently is sensitive to cyanide. Henry and Nyns (1975) listed only two species, *Euglena gracilis* (also classified as a protozoan) and *Nitella clavata*, which are able to develop a distinct mitochondrial insensitive respiration.

One of the terminal oxidases of the green alga *Chlorella vulgaris* was not inhibited by 1 m*M* cyanide or 0.1 m*M* thiocyanate (Sargent and Taylor, 1972). This enzyme differed from the typical cytochrome oxidase in that it had about one-fourth the capacity for oxygen uptake and was resistant to cyanide.

Although CCCP (Section 3.3.2.1.3) apparently acts as an uncoupler, recent data with *Chlorella vulgaris* suggest that degradation of CCCP also can lead to a significant production of HCN (Pistorius et al., 1975). Additional information is necessary to determine if the uncoupling properties of CCCP are due in all cases to cyanide production.

3.3.2.2 Nucleic Acid Metabolism — In *E. coli* cyanide blocks the propagation of the replicating fork of the circular chromosome (Olivera and Lundquist, 1971). The extremely rapid inhibition is noticeable in less than one-thousandth of the generation time (Cairns and Denhardt, 1968). These KCN-inhibited bacteria could initiate DNA synthesis after being infected with bacteriophage ϕX174. Nazar and Wong (1969) reported that 25 μ*M* cyanide produced immediate inhibition of RNA synthesis in *E. coli* strain 15T$^-$ but did not affect DNA synthesis. With a higher concentration, Olivera and Lundquist (1971) found that 0.5 m*M* KCN caused an immediate inhibition of thymine incorporation into the DNA of exponentially growing *E. coli* strain 15T$^-$. After a 1-hr lag period, however, incorporation resumed and eventually growth occurred (Figure 3.11). Weigel (1974) found that KCN decreased ATP and deoxynucleoside triphosphate (dNTPs) concentrations within a few seconds in conjunction with the rapid decrease in DNA replication. This decrease in ATP and dNTPs is presumably responsible for the decrease in replication (Weigel and Englund, 1975). The site of cyanide action is apparently complex because both aerobically and anaerobically grown cells are inhibited. Thus, inhibition is not due only to inhibition of electron transport but is "due to the ability of this highly reactive compound to react non-specifically with many proteins and

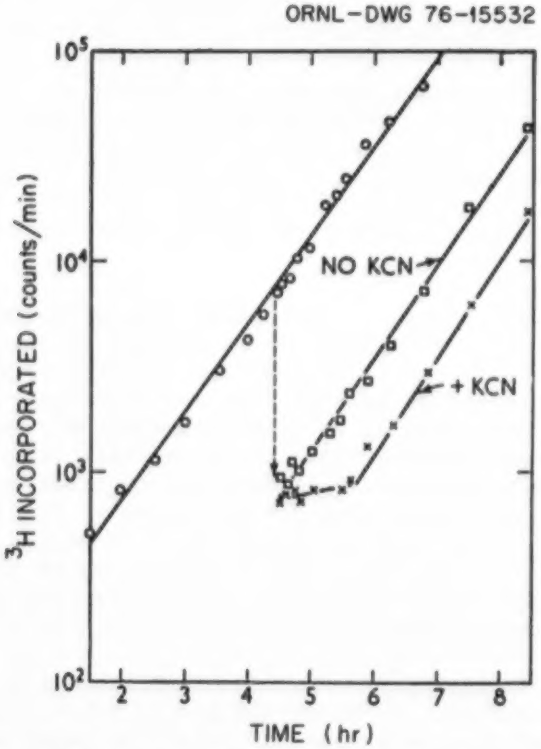

Figure 3.11. Inhibition of DNA synthesis by cyanide. Source: Adapted from Olivera and Lundquist, 1971, Figure 1, p. 266. Reprinted by permission of the publisher.

other molecules." In contrast to the data of Olivera and Lundquist (1971), Weigel and Englund (1975) did not observe a recovery from KCN inhibition.

Chlamydomonas reinhardi cells treated with the DNA-methylating agent methyl methanesulfonate (MMS) exhibited a rapid drop in survival when exposed to 10^{-3} to 10^{-2} *M* KCN (Loppes, 1967). The survival of untreated cells did not decrease when exposed to these same cyanide concentrations. If the MMS-treated cells were incubated overnight in buffer prior to cyanide addition, the cyanide sensitivity was almost completely lost. The cyanide posttreatment was thought to inhibit some respiratory processes involved in repair of DNA damage by MMS so that repair could not proceed.

The only data available for cyanide effects on viruses were concerned with the formation of an HCN-photoproduct upon ultraviolet irradiation of tobacco mosaic virus RNA in the presence of HCN (Borazan, 1973). The photoproduct chromatographed similarly to a known uridine-HCN photoproduct. HCN was weakly bonded to the RNA with or without irradiation and most HCN could be removed by washing. However, a residual amount always remained complexed. The author suggested that the bond might involve a trace metal within the RNA.

Wagner et al. (1950) used KCN as a mutagen to produce biochemical mutants of *Neurospora crassa* (conidia treatment). Mutation rates were less than those obtained by direct ultraviolet irradiation.

Radioprotective effects against x-irradiation in *E. coli* B have been produced by malononitrile, methyl malononitrile, benzal malononitrile, the 4-chloro- and 4-oxy- derivatives of benzal malononitrile, acetonitrile, propionitrile, butyronitrile, ethyl malononitrile, cyanoacetamide, and 1,1,6,6-tetracyano-2,5-dimethyl-1,5-hexadien (Hernádi et al., 1968). Of the organic sulfocyanide derivatives tested, none were effective radioprotectors.

3.3.2.3 Amino Acid Transport — Amino acid transport can be inhibited by cyanide. Examples of such inhibition were found in bacterial systems. Glycine and L-serine transport by membrane vesicles of *Thiobacillus neapolitanus* was completely inhibited by 10 m*M* cyanide (Matin et al., 1974). In *Pseudomonas putida*, L-lysine transport was inhibited 95% by 17 m*M* KCN (Miller and Rodwell, 1971). In membrane vesicles of *E. coli* ML308-225, 2 m*M* NaCN inhibited D-lactate-driven transport of proline by 50% and internally generated NADH-driven transport by 25% (Futai, 1974). A concentration of 10 m*M* NaCN inhibited these processes by 88% and 87% respectively. Ferricyanide (0.5 to 4.0 m*M*) inhibited transport stimulated by additions of NADH to the medium but did not inhibit transport stimulated by internally generated NADH.

3.3.2.4 Enzymatic Activities — Cyanide inhibits many enzymatic reactions involved in processes such as hemolysis and nitrogen cycling. In 12 strains of *E. coli* isolated from intestines of pigs with edema disease, 40 μ*M* KCN inhibited β-hemolysis 80% to 90% (Short and Kurtz, 1971). Complete inhibition was obtained with 400 μ*M* KCN. Cells were tested in early, middle, and late exponential growth periods. Cyanide did not inhibit the β-hemolysis of cell-free supernatants.

Cyanide can also affect nitrogen metabolism. For example, Schloemer and Garrett (1974) found that KCN (1 m*M*) decreased nitrite transport in *Neurospora crassa* by inhibiting nitrite reductase by 95%; the inhibition appeared to be reversible. The enzyme NADH—nitrate oxidoreductase of *Chlorella vulgaris* is converted in the presence of NADH and cyanide to an inactive form which can be reactivated by ferricyanide (Lorimer et al., 1974; Solomonson, 1974; Solomonson and Vennesland, 1972). The cyanide binding was directly proportional to the amount of enzyme inactivation (0.066 nmole of cyanide per unit of enzyme inactivated) (Lorimer et al., 1974). Pistorius et al. (1975) reported that *C. vulgaris* produced HCN at high light intensities, high O_2 tension, and low CO_2 tension. Although there is no direct evidence, Lorimer et al. (1974) stated that the cyanide inactivation of nitrate reductase in *C. vulgaris* may be a natural control mechanism to inactivate that enzyme when ammonium is the nitrogen source. Vennesland and Jetschmann (1976) suggested that the production and excretion of glycolate by illuminated cultures of *C. vulgaris* at high O_2 and low CO_2 tensions may be regulated by internal HCN production. Externally added cyanide, hydroxylamine, hydrazine, or semicarbazide were able to stimulate glycolate synthesis. Nitrate reductase A from *Aerobacter aerogenes* and *Micrococcus denitrificans* is strongly inhibited by cyanide (Pichinoty, 1969). This inhibition is not completely reversible. Approximately 50% of the enzyme activity could be restored when nitrate and chlorate were used as substrates. In *M. denitrificans* and *P. aeruginosa*, *p*-phenylene-diamine-NO_2^- reductase was also inhibited by cyanide (Pichinoty, Bigliardi-Rouvier, and Rimassa, 1969). Nitrate reductase from *Nitrobacter agilis* inactivated by NADH could be reactivated by 0.5 m*M* ferricyanide (Herrera and Nicholas, 1974).

Various pesticides were found to stimulate the growth of *Azotobacter vinelandii* in culture (Peeters et al., 1975). Calcium cyanide, however, decreased growth in culture (Table 3.8). Most of these pesticides decreased the amount of nitrogenase extracted from these cells. Data on the inhibition of the isolated enzyme by a few of these pesticides are given in Table 3.9 (data for calcium cyanide was not reported).

Other microbial enzymes are inhibited by cyanide. For example, 1 m*M* NaCN inhibited purified δ-aminolaevulate dehydratase from photosynthetically grown *Rhodopseudomonas spheroides* (van Heyningen and Shemin, 1971). The enzyme CO_2 reductase from *Chlostridium pasteurianum* ATCC 6013 was inactivated by 10^{-4} *M* cyanide (Thauer et al., 1973).

Cyanogen bromide is often used to characterize the active site of an enzyme and apparently cleaves a polypeptide chain by reacting with methionine (Lehninger, 1970). Examples include analyses of trypsin from *Streptomyces* (Olafson et al., 1975) and of invertase from *Saccharomyces cerevisiae*. This compound, however, is apparently not of environmental concern.

TABLE 3.8. INFLUENCE OF VARIOUS PESTICIDES ON THE YIELD OF BACTERIAL MASS OF *AZOTOBACTER VINELANDII* AND THE SYNTHESIS OF NITROGENASE ENZYME COMPLEX

Compound	Applied dose (g/10 liters)	Yield of wet bacteria (g/10 liters of medium)	Specific activity[a]
None		165	5
Ammonium thiosulfate[b]	1.2	474	0
Calcium cyanide[b]	0.2	74	0
Potassium cyanate[b]	1.2	174	0.04
Cyanuric acid[c]	Saturated	200	0.26
Nirit Supra (2,4-dinitrophenyl thiocyanate)[c]	1.3	394	0.5

[a] Specific activity of nitrogenase enzyme complex in nanomoles of C_2H_4 per minute per milligram of protein.

[b] Soluble in culture medium.

[c] Poorly soluble in culture medium.

Source: Adapted from Peeters et al., 1975, Table II, p. 405. Reprinted by permission of the publisher.

TABLE 3.9. EXTENT OF INHIBITION OF SPECIFIC ACTIVITY OF NITROGENASE ENZYME COMPLEX OF *AZOTOBACTER VINELANDII* BY SEVERAL PESTICIDES

Compound	Use	Concentration (m*M*)	Inhibition[a] (%)
Ammonium thiocyanate[b]	Herbicide	3.5	30
Potassium cyanate[b]	Herbicide	3.5	30
Cyanuric acid[b]	Insecticide	0.1-1	0
Nirit Supra (2,4-dinitrophenyl thiocyanate)[c]	Fungicide	0.7	50

[a] Percent inhibition is indicated at the concentration given in the concentration column. When no inhibition is observed, the range of concentration tested is given.

[b] Soluble in water.

[c] Soluble in dimethyl sulfoxide.

Source: Adapted from Peeters et al., 1975, Table IV, p. 406. Reprinted by permission of the publisher.

SECTION 3

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SECTION 4

BIOLOGICAL ASPECTS IN PLANTS

4.1 SUMMARY

Free cyanide is not found in intact plant cells. Many plant species, such as cassava, sorghum, flax, cherries, almonds, and lima beans, contain cyanogenic glycosides which release hydrocyanic acid (HCN) when they are hydrolyzed. It should be pointed out that cyanogenic compounds are far more widely distributed than any of the other nitrile compounds discussed in this section. These glycosides are biosynthesized from amino acids and sugars. The amounts formed depend on the physiological status of the plant; younger plants and tissues often have a higher glycoside content than mature parts. Glycosidase, the enzyme responsible for glycoside hydrolysis, is usually compartmentalized within the cell and contacts the substrate only when the plant is bruised or crushed. Free HCN may be released by living roots of sorghum, but other cyanogenic plants are not known to possess this ability. The production and release of cyanide to the environment through normal processes of death and decomposition have not been studied.

Other plant species neither contain cyanide nor release it upon injury. The Cruciferae contain glucosinolates (mustard oil glycosides) which hydrolyze when the cell is injured and release isothiocyanates, thiocyanates, or organic nitriles. These glucosinolates are synthesized from amino acids and sugars. Within the Cruciferae, indoleacetonitrile may be a natural metabolite involved in synthesis of indoleacetic acid and/or glucobrassicin (a glucosinolate).

Although no definitive evidence on whether HCN fixation is ubiquitous in all plants has been shown, some plants possess the ability to metabolize externally added HCN. The major route appears to be condensation of cyanide with cysteine to yield β-cyanoalanine, which is subsequently hydrolyzed to asparagine. The synthesis of asparagine by this pathway is probably of minor importance in total asparagine biosynthesis. In common vetch, β-cyanoalanine condenses with glutamic acid to produce a lathyrogenic compound, γ-glutamyl-β-cyanoalanine. In *Lathyrus*, β-cyanoalanine is converted to β-aminopropionitrile and then is condensed with glutamic acid.

Cyanopyridine alkaloids, natural compounds of limited distribution in the plant kingdom, contain the cyanide group but do not release cyanide during either synthesis or degradation. Pseudocyanogenic glycosides, natural compounds which are limited to the Cycadaceae, release HCN only under the condition of alkaline hydrolysis.

The major effect of cyanide on metabolism is through the inhibition of respiration by complexation of iron in cytochrome oxidase. In some species, cyanide inhibition of respiration is not complete and the proportion that is insensitive varies with plant organ, ageing, and physiological status of the tissue. Details of the electron transport chain for cyanide-insensitive respiration are unknown, but this chain apparently involves only one site for adenosine triphosphate (ATP) synthesis.

In vitro cyanide can inhibit a variety of enzymes by complexation of metal cofactors, by combination of undissociated HCN with carbonyl groups, or by combination of the cyanide ion with disulfide bonds. The significance of these reactions in vivo is unknown.

Because cyanide inhibits respiration, and hence, ATP production, it also inhibits a variety of processes directly or indirectly dependent on ATP (e.g., ion uptake and translocation in phloem). However, in some species cyanide can stimulate germination — perhaps by increasing the flow of carbon through the pentose phosphate pathway. Cyanide in the presence of oxygen has been observed to increase chromosomal aberrations in broad bean roots.

Nitrile herbicides act as electron flow inhibitors or uncouplers in respiration and/or photosynthesis. No release of cyanide from these compounds has been observed. Dichlobenil is a preemergent herbicide; ioxynil and bromoxynil are postemergent contact herbicides.

4.2 METABOLISM

The cyanide group is present in a variety of natural organic compounds. The major group of these compounds is the cyanogenic glycosides, which are rather widely distributed within the plant kingdom (Conn, 1969). The metabolism of these glycosides is considered in some detail in this section; damage (bruising, crushing, etc.) to cells of plants containing these compounds causes enzymatic release of hydrocyanic acid (HCN).

The metabolism of nitrile herbicides is briefly discussed. Herbicidal activity apparently is not related to the formation of free HCN and, where studied, metabolism of these herbicides does not involve HCN release.

4.2.1 Hydrocyanic Acid Incorporation

Although little or no free HCN occurs in plants (Robinson, 1975), plants are able to metabolize externally added HCN. Blumenthal-Goldschmidt, Butler, and Conn (1963) observed that the cyanogenic plants sorghum, flax, and white clover converted $H^{14}CN$ to the amide-carbon atom of asparagine. However, the ability to fix HCN was not related to the cyanogenic nature of these plants. Barley, pea, and red clover, none of which contain cyanogenic glycosides, also convert HCN to asparagine.

Blumenthal-Goldschmidt, Butler, and Conn (1963) initially proposed that β-cyanoalanine was an intermediate in the conversion of HCN to asparagine and that it was formed enzymatically by the reaction of HCN with serine. In a preliminary enzyme study, however, cysteine was a better substrate than serine for asparagine production (Floss, Hadwiger, and Conn, 1965). Subsequently, the enzyme β-cyanoalanine synthetase, which catalyzes the reaction

$$\text{cysteine} + \text{HCN} \rightarrow \beta\text{-cyano-L-alanine} + H_2S\ ,$$

was isolated (Hendrickson and Conn, 1969). This enzyme is responsible for the production of β-cyano-L-alanine in common vetch, lupine, and sorghum (Blumenthal-Goldschmidt et al., 1968), in several other vetches — *Lathyrus* *odoratus*, *Ecballium elaterium*, and *Chlorella pyrenoidosa* (Fowden and Bell, 1965), in *Escherichia coli* (Dunnill and Fowden, 1965), as well as in numerous other plants that convert HCN to asparagine (Conn and Butler, 1969).

In common vetch (*Vicia sativa*) most HCN was converted into an unknown compound instead of asparagine (Blumenthal-Goldschmidt, Butler, and Conn, 1963); this unknown compound was later shown by Ressler, Giza, and Nigam (1963) to be γ-L-glutamyl-β-cyano-L-alanine. Fowden and Bell (1965) then demonstrated that common vetch and *C. pyrenoidosa* contained a glutamyl transferase that added glutamic acid to β-cyano-L-alanine and that this transferase was absent in other *Vicia* species. Since a majority of the species which metabolize HCN by the β-cyano-L-alanine pathway produce asparagine instead of the glutamvl peptide, they must lack the transferase and contain instead an enzyme that converts β-cyanoalanine to asparagine. Castric, Farnden, and Conn (1972) have partially purified an enzyme from blue lupine which catalyzes this reaction. The plant enzyme is distinct from the asparaginase of *E. coli*, which does catalyze the conversion of β-cyanoalanine to asparagine (Jackson and Handschumacher, 1970).

β-Cyanoalanine synthetase occurs in the mitochondrial fraction of plant seedlings (Floss, Hadwiger, and Conn, 1965; Hendrickson and Conn, 1969) and reaches a maximal concentration in five-day-old seedlings of *Lupinus angustifolius* (Lever and Butler, 1971). Since the maximum enzyme activity occurred prior to maximal accumulation of asparagine, the latter authors concluded that the β-cyanoalanine pathway was not a major route for asparagine biosynthesis in the blue lupine. Other authors (Castric, Farnden, and Conn, 1972; Oaks and Johnson, 1972) who have examined the β-cyanoalanine pathway in other plants have also reached the same conclusion.

The physiological significance of the β-cyanoalanine pathway for HCN metabolism is not clear. Abrol and Conn (1966) and Abrol, Conn, and Stoker (1966) presented evidence that endogenous turnover of cyanogenic glycosides in lotus and in *Nandina domestica* Thunb. releases HCN, which is then incorporated into asparagine. In those plants that do not produce cyanogenic glycosides, the situation is more puzzling. The enzyme system for metabolizing HCN is present, but the endogenous source of HCN is unknown. Conn and Butler (1969) have suggested that the β-cyanoalanine pathway is a metabolic activity acquired early in evolution and retained by species that no longer have a need for such a process.

A few plants apparently release HCN to the environment through normal metabolism as opposed to release during destructive actions such as bruising or crushing. Sorghum roots released free HCN — about 0.005 mg per plant per 24 hr for one variety and about 0.02 mg per plant per 24 hr for another variety (Rangaswami and Balasubramanian, 1963). The occurrence of this ability in the plant kingdom and the ecological significance of HCN release have not been well studied.

Cyanides and organic nitriles can react with nitrogenase, the enzyme complex responsible for nitrogen fixation (Burns and Hardy, 1975). The reaction involves the cleavage of the C≡N bond and produces ammonia and the corresponding hydrocarbon. Cyanide probably does not undergo a reaction with nitrogenase within the cell because of its high affinity for metalloproteins. Organic nitriles have varying reactivities with nitrogenase, depending upon the nature of the hydrocarbon position. There is little information on the in vivo significance of such reduction.

4.2.2 Cyanide Release from Plants

The effects of the natural release of HCN from sorghum roots on the neighboring biota have not been studied. Rangaswami and Balasubramanian (1963) reported that sorghum roots released HCN and suggested that this release may be responsible for the sparsity of serious fungal or bacterial root diseases in sorghum. The isolation of an HCN-resistant *Aspergillus niger* strain from the sorghum rhizosphere, not from the soil itself, could indicate that HCN is rapidly metabolized in the rhizosphere and, thus, is not available for toxic action.

The relationships among HCN, cyanogenic glycosides, and disease resistance in plants have been the subject of several studies. Reynolds (cited in Timonin, 1941) correlated the resistance of flax to *Fusarium lini* with the HCN "recovered from plant tissues." Timonin (1941) presented evidence that HCN was produced and excreted by the roots of a wilt-resistant flax variety but not by a susceptible variety. Presumably, the HCN was released by the hydrolysis of the cyanogenic glycoside linamarin. Millar and Higgins (1970) showed that HCN was released from the cyanogenic glycosides linamarin and lotaustralin during the infection of bird's-foot trefoil with *Stemphylium loti*. This fungus was more resistant to HCN than were other fungi which were not parasitic to bird's-foot trefoil. *S. loti* also produced a β-glucosidase which could hydrolyze the cyanogenic glycosides from a trefoil strain that did not possess an endogenous β-glucosidase.

Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and its derivatives are effective uncouplers of oxidative and photosynthetic phosphorylation and are used in biochemical analysis (Pistorius et al., 1975). When CCCP was added to spinach grana or *Chlorella* cultures, cyanide was released; the amount released was stimulated by light and oxygen. Presumably, the cyanide was derived from the cyano group of CCCP. Further work is necessary to determine if this cyanide generation is responsible for the uncoupling activity of CCCP.

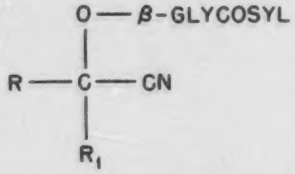
4.2.3 Cyanogenic Glycosides

4.2.3.1 Catabolism and Anabolism of Cyanogenic Glycosides — In higher plants, the major group of compounds that contains the cyanide group is the cyanogenic glycosides (Robinson, 1975). These compounds have been identified in approximately 1000 plant species, comprising 90 families and 250 genera (Conn, 1969). No specific comments on the evolutionary relationships among these families were found in the literature, although Alston and Turner (1963) stated that "no clear cut systematic implications are evident." These compounds are quite common in certain families. For example, cyanogenic glycosides occur in 150 species of Rosaceae, 100 species of the Leguminosae, 100 species of the Gramineae, 50 species of the Araceae, 50 species of the Compositae, and lesser numbers of the Euphorbiaceae, Passifloraceae, Ranunculaceae, and Saxifragaceae (Alston and Turner, 1963). Within these families, cyanogenic glycosides can be used to determine systematic relationships. Alston and Turner (1963) cited the work of Gibbs and Hegnauer to demonstrate that "cyanogenesis" (defined as the release of cyanide from plants; whether the cyanide is actually released from cyanogenic glycosides is usually not determined) is common in the Rosaceae subfamilies Pomoideae and Prunoideae but not in the Rosideae or Spiraeoideae. Characterization of the particular cyanogenic glycoside in each species is essential for further systematic studies.

Because of the occurrence of cyanogenic glycosides in a variety of foods consumed by humans and the subsequent release of HCN during cell destruction, care must be taken in the preparation of these foods to avoid cyanide poisoning (Conn, 1973*a*; Montgomery, 1969). This problem is more critical in parts of the world where cyanogenic plants (e.g., cassava) constitute a major portion of the diet. Some food plants containing cyanogenic glycosides and thus having a cyanide-releasing potential are almonds, cassava, lima beans, macadamia nuts, bamboo shoots, numerous stone fruits, sorghum, and corn.

Poisoning of livestock by cyanogenic plants is also a problem which has occurred in various countries (Kingsbury, 1964). In the United States, cattle have been poisoned by eating various species of *Sorghum* (grain sorghum, Sudan grass, Johnson grass). Under dry growing conditions, arrowgrass (*Triglochin maritima* and *Triglochin palustria*) can develop concentrations of cyanogenic glycosides which release enough cyanide to poison sheep and cattle (Radeleff, 1970).

Approximately 20 different cyanogenic glycosides are known and all have the basic structure:



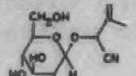
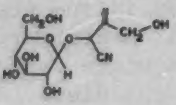
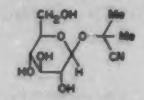
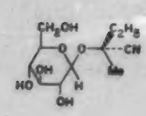
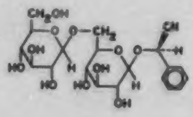
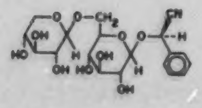
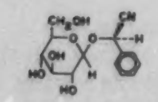
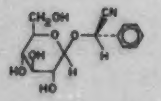
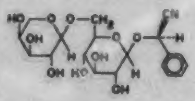
$$R-\overset{\displaystyle O-\beta\text{-GLYCOSYL}}{\underset{\displaystyle R_1}{C}}-CN$$

where R and R_1 refer to hydrogen, alkyl, or aryl groups.

Table 4.1 lists the major cyanogenic glycosides and representative species in which they occur. The particular cyanogenic glycoside in many plant species has not been identified and many possibilities for substitution within the general formula exist; therefore, additional glycosides may be discovered and characterized. For example, Seigler et al. (1975) reported the isolation of dihydroacacipetalin, a new cyanogenic glycoside from *Acacia sieberiana* var. *woodii*, and Tantisewie, Ruijgrok, and Hegnauer (1969) described deidamin, a newly characterized cyanogenic glycoside from *Deidamia clematoides* (Passifloraceae).

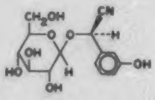
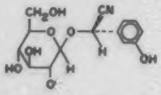
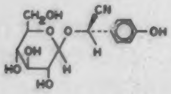
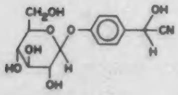
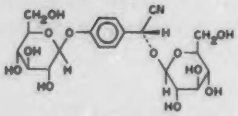
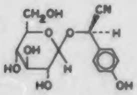
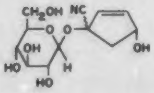
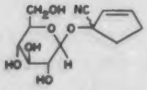
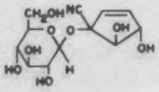
The cyanogenic glycosides themselves are not highly toxic to animals. Hydrolysis of the cyanogenic glycoside releases HCN, which is the toxic agent in cyanophoric plants. Hydrolysis occurs when the plant cells are injured, allowing previously compartmentalized hydrolytic enzymes to

TABLE 4.1. NATURALLY OCCURRING GLYCOSIDES

Name	Formula	Source
	Derivatives of valine, isoleucine, and leucine	
Acacipetalin		*Acacia stolonifera* Burch.
Cardiospermin		Sapindaceae
Linamarin		*Manihot utilissima* Pohl (cassava), *Linium usitatissimum* L. (flax), *Phaseolus lunatus* L. (lima bean), *Hevea brasiliensis* Muell., *Trifolium repens* L.
Lotaustralin		Same as Linamarin
	Derivatives of phenylalanine	
(R)-Amygdalin		*Prunus amygdalus* Stokes, many other Rosaceous species
(R)-Lucumin		Sapotaceae
(R)-Prunasin		*Prunus padus* L., many other species from a variety of families
(S)-Sambunigrin		*Sambucus nigra* L., *Acacia cunninghamii*
(R)-Vicianin		*Vicia angustifolia* Roth L.

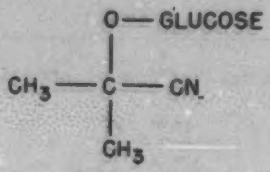
(continued)

TABLE 4.1 (continued)

Name	Formula	Source
Probable derivatives of phenylalanine		
(R)-Holocalin		Leguminosea, Caprifoliacea
(S)-Zierin		*Zieria laevigata* Sm.
Derivatives of tyrosine		
(S)-Dhurrin		*Sorghum vulgare* Pers., *Phyllanthus gasstroemi* Muell.
p-Glucosyloxymandelonitrile		Berberidaceae, Ranunculaceae, *Goodia latifolia* Salib.
(R)-Proteacin		Proteaceae, Ranunulaceae
(R)-Taxiphyllin		*Taxus baccata*
Compounds with cyclopentene ring structure		
Barterin (tetraphyllin B)		May occur in Flacourtiaceae and Passifloraceae
Deidaclin		May occur in Flacourtiaceae and Passifloraceae
Gynocardin		*Gynocardia odorata* R. Br., *Pangium edule* Reinw.

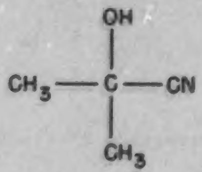
Source: Modified from Seigler, 1975 and Conn, 1969.

degrade the glycosides. Hydrolysis usually occurs in two steps. For instance, linamarin,



$$\mathrm{CH_3-\overset{O-GLUCOSE}{\underset{CH_3}{C}}-CN}$$

is first hydrolyzed by a β-glucosidase, linamarase, to yield glucose and acetone cyanohydrin,



$$\mathrm{CH_3-\overset{OH}{\underset{CH_3}{C}}-CN}$$

The cyanohydrin is then cleaved by an oxynitrilase to yield acetone and HCN (Conn, 1969, 1973*a*). Some plants containing cyanogenic glycosides do not possess the necessary degradative enzymes (Robinson, 1975), but this is the exception rather than the rule.

The glucosidases isolated from a given species have a certain amount of substrate specificity. For example, flax glucosidase hydrolyzes linamarin, which is found in flax, and the chemically related lotaustralin, found in *Lotus* sp. and flax, but not amygdalin found in *Prunus* sp. The oxynitrilases from different species also exhibit varying degrees of substrate specificity (Conn, 1969).

Biosynthetic pathways for cyanogenic glycosides involve the conversion of specific protein amino acids to the corresponding aldoximes and their subsequent conversion to the nitrile. The nitrile is hydroxylated and the sugar moiety is then attached (Figure 4.1). Thus, L-tyrosine is ultimately converted to dhurrin and toxiphyllin; L-phenylalanine to prunasin, amygdalin, and vicianin; L-valine to linamarin; and L-isoleucine to lotaustralin (Conn, 1973*b*). Feeding of radioactively labeled amino acids to plants (e.g., valine and isoleucine to flax) or of labeled hydroxynitriles (acetone cyanohydrin or butanone cyanohydrin fed to flax) results in the formation of labeled cyanogenic glycosides (linamarin and lotaustralin in flax) (Hahlbrock and Conn, 1971). In the previous example, both glycosides are apparently formed in vivo by the same glycosyltransferase.

The physiological role of cyanogenic glycosides in plants is not known. However, some workers have postulated their involvement in the coevolution of plants and insects (Jones, 1973). They are apparently actively metabolized (Abrol and Conn, 1966) and do not represent metabolic end products.

4.2.3.2 Content of Cyanogenic Glycosides in Various Plants — The relationship between the potential for producing hydrocyanic acid (HCN-p) and various factors has been well studied in the diverse genus *Sorghum* because of its economic importance as a crop and the threat of livestock poisoning.

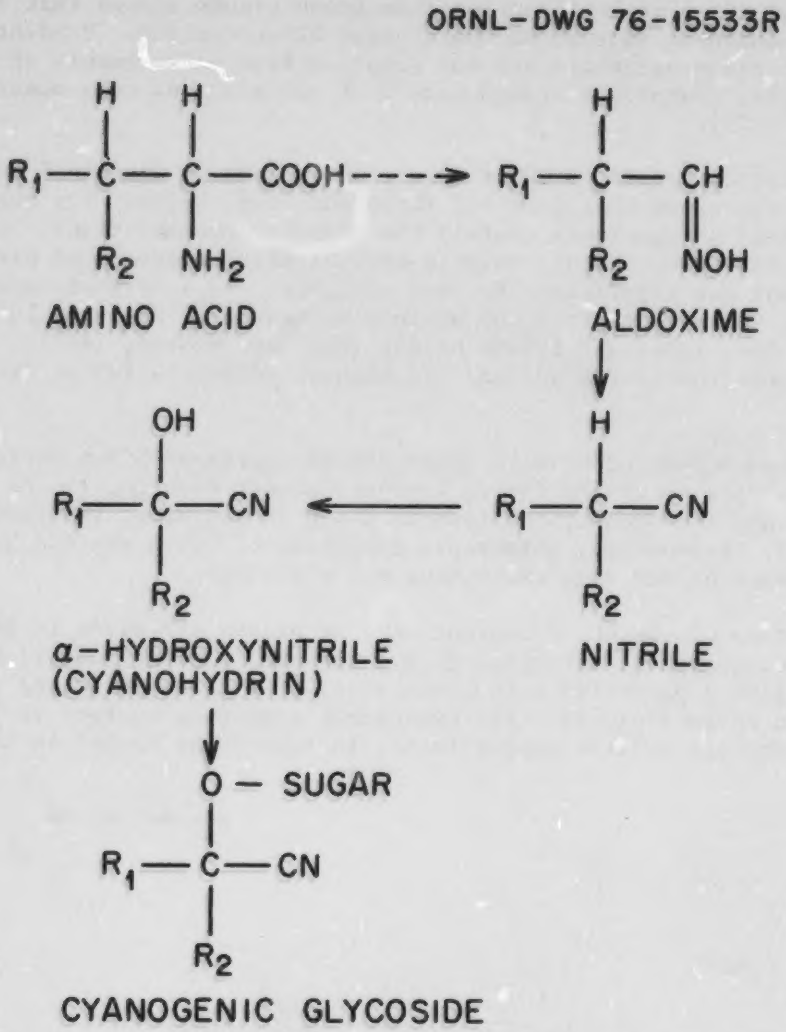

ORNL-DWG 76-15533R
AMINO ACID
ALDOXIME
NITRILE
α-HYDROXYNITRILE (CYANOHYDRIN)
CYANOGENIC GLYCOSIDE
R1
R2
COOH
NH2
CH
NOH
H
CN
OH
O – SUGAR

Figure 4.1. Generalized biosynthetic pathway for cyanogenic glycosides. Source: Adapted from Conn, 1969, Figure 11, p. 525. Reprinted by permission of the publisher.

Sorghum genotypes all contain dhurrin as a precursor of HCN. Harrington (1966) reviewed earlier work and concluded that "(1) HCN-p varies with species and cultivar, (2) HCN-p decreases as plant height and age increase, (3) HCN-p varies with location and climate, (4) moisture stress results in higher HCN-p and (5) HCN-p is higher in first growth than in aftermath growth."

An easy screening procedure is necessary to select cultivars with low HCN-p. Whole plants of *Sorghum* variety Piper had an average content of about 12 to 17 ppm HCN-p (fresh weight basis), while the variety Suhi-1 averaged about 70 to 80 ppm HCN-p (Benson, Gray, and Fribourg, 1969). An analysis of HCN-p of leaf samples from these plants showed that leaf samples are not a suitable measure of whole plant HCN-p content. Prediction of HCN-p of sorghum varieties was not possible from measurements of various morphological features although some weak correlations were observed (James and Gray, 1975).

The concentration of HCN-p in various organs of Greenleaf Sudan grass (sorghum) are shown in Figure 4.2 (Loyd and Gray, 1970). Dry seeds contain little HCN-p; young plants contain the highest concentrations. Correlations between total HCN-p and dry weights of individual aboveground parts were positive but not significant for any cultivar. In a sorghum—Sudan grass hybrid the HCN-p content of the whole plant was 372, 254, and 204 ppm for plants of 50-, 120-, and 155-cm height (Wolf and Washko, 1967). At all three heights the leaf blade had the highest concentration of the parts studied.

Sorghum subjected to mild frost showed increased HCN-p content within one to six days after the frost; however, severe freezing led to death of the plant and a concommittant loss in HCN-p of the plant (Wattenbarger et al., 1968). Presumably, this rapid depletion of HCN-p was due to the enzymatic release of HCN from the cyanogenic glycoside.

Examples of the HCN-p content of some plants are given in Table 4.2. Contents of different varieties (e.g., cassava) can vary considerably. DeBruijn (1971) presented a thorough study of factors affecting the cyanide content in cassava plants. The cyanogenic glycoside content of leaves decreased with age and the concentration in tubers was higher in the bark than

ORNL-DWG 76-15534

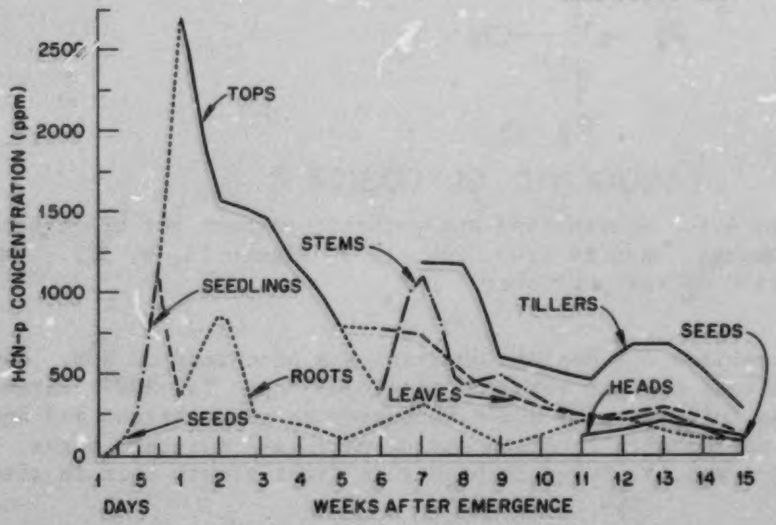


Figure 4.2. Concentration of hydrocyanic acid potential in plant parts of Greenleaf Sudan grass (sorghum). Source: Adapted from Loyd and Gray, 1970, Figure 2, p. 395. Reprinted by permission of the publisher.

TABLE 4.2. CYANIDE CONTENT OF SELECTED PLANTS

Plant material	Cyanide content	Reference
Bitter almond	280 ppm	György et al., 1969
	250 mg/100 g	Montgomery, 1969
Spicy almond	86-98 ppm	György et al., 1969
Sweet almond	22-54 ppm	György et al., 1969
Peach leaves		
var. Alexander	39 ppm	György et al., 1969
var. Mariska	74 ppm	György et al., 1969
var. Ford	120 ppm	György et al., 1969
Cassava roots		
var. Palmeiras	378 ppm	Esquivel and Maravalhas, 1973
var. CEPEC 62	313 ppm	Esquivel and Maravalhas, 1973
var. IAC 780	200 ppm	Esquivel and Maravalhas, 1973
var. Itapecuru	68 ppm	Esquivel and Maravalhas, 1973
var. Engole boi	41 ppm	Esquivel and Maravalhas, 1973
var. CEPEC	27 ppm	Esquivel and Maravalhas, 1973
Bitter cassava		
dried root cortex	245 mg/100 g	Montgomery, 1969
stem	113 mg/100 g	Montgomery, 1969
whole root	55 mg/100 g	Montgomery, 1969
Sorghum, young plant	250 mg/100 g	Montgomery, 1969
Bamboo		
tip	800 mg/100 g	Montgomery, 1969
stem	300 mg/100 g	Montgomery, 1969
Lima bean		
Java	312 mg/100 g	Montgomery, 1969
Puerto Rico	300 mg/100 g	Montgomery, 1969
Burma	210 mg/100 g	Montgomery, 1969
Arizona	17 mg/100 g	Montgomery, 1969
America	10 mg/100 g	Montgomery, 1969
European contoneaster fruit	1.08 mg/100 g frozen wt	Jeffrey and Wiebe, 1971
Hedge contoneaster fruit	2.75 mg/100 g frozen wt	Jeffrey and Wiebe, 1971
Miniature crab apple fruit	1.70 mg/100 g frozen wt	Jeffrey and Wiebe, 1971
American mountain ash fruit	1.80 mg/100 g frozen wt	Jeffrey and Wiebe, 1971
European mountain ash fruit	6.47 mg/100 g frozen wt	Jeffrey and Wiebe, 1971

in the inner sections. Concentrations varied considerably among tubers on the same plant, but no correlation with tuber size was evident. Nitrogen additions to soil increased the glycoside content in leaves and roots, whereas additions of potassium and "farmyard manure" decreased the content. In pot experiments, drought increased the glycoside content of young plants. In field experiments, however, a dry season in three areas did not increase the glycoside content in roots. These and other observations indicate that the final glycoside content at a given time or developmental stage is the result of various interacting physiological processes.

4.2.4 Pseudocyanogenic Glycosides

Pseudocyanogenic glycosides, a series of toxic compounds found primarily in members of the Cycadaceae, have the formula $CH_3{-}N{=}N{-}CH_2{-}O{-}$(sugar) (Miller, 1973). Although acid or enzymatic hydrolysis of these compounds does not produce free HCN, treatment with cold alkali will release HCN; for example:

$$\text{macrozamin} \xrightarrow{\text{acid}} N_2 + CH_3OH + HCHO + \text{primeverose} ,$$

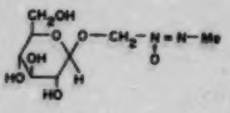
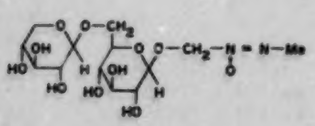
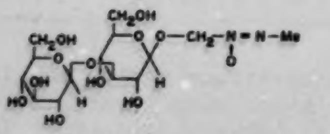
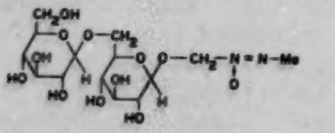
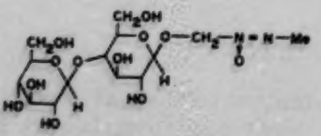
$$\text{macrozamin} \xrightarrow{\text{cold NaOH}} N_2 + HCN + HCOOH + \text{primeverose} .$$

Table 4.3 lists the common pseudocyanogenic glycosides (Seigler, 1975). These compounds have the same nitrogenous moiety but differ in the constituent sugar.

4.2.5 Lathyrogenic Compounds

Certain compounds that produce distinct maladies in animals ingesting them are called lathyrogenic compounds because they were initially found in the genera *Lathyrus* and *Vicia*. For example, γ-glutamyl-β-aminoproprionitrile is produced in *Lathyrus* and γ-glutamyl-β-cyanoalanine in *Vicia sativa*. In *Lathyrus*, β-cyanoalanine (formed by HCN reacting with cysteine) is converted to β-aminopropionitrile, which then condenses with glutamic acid (Ferris, 1970). β-Cyanoalanine is not converted to asparagine in *V. sativa* because of the lack of the hydrolase enzyme (Castric, Farnden,

TABLE 4.3. PSEUDOCYANOGENIC GLYCOSIDES

Name	Formula[a]	Source
Cycasin		*Cycas revoluta* Thunb.
Macrozamin		*Macrozamia spiralis* Miq., *M. reidlei* C. Cl. Gardner
Neocycasin A		*Cycas revoluta*
Neocycasin B		*Cycas revoluta*
Neocycasin E		*Cycas revoluta* seeds

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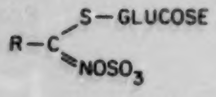
[a] The position of the oxygen in the azoxy group is not established.

Source: Modified from Seigler, 1975 and Miller, 1973.

and Conn, 1972) and is therefore available for other conversions such as the addition of the glutamyl group. Another neurolathyrogenic factor, α,γ-diaminobutyric acid, is produced in *V. sativa* and *L. odoratus* presumably by γ-reduction of β-cyanoalanine. These compounds are apparently formed by normal metabolism and not in response to exposure to cyanide in the environment.

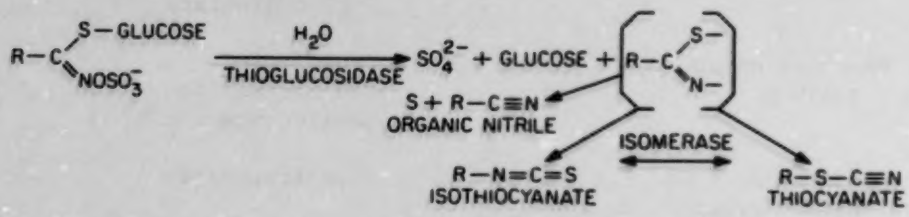
4.2.6 Glucosinolates (Thioglucosides)

Glucosinolates have the general formula



$$R-C\begin{matrix}\nearrow S-\text{GLUCOSE}\\ \searrow \text{NOSO}_3\end{matrix}$$

and upon enzymatic hydrolysis during crushing of the plant, isothiocyanates or thiocyanates are rapidly formed (VanEtten, Daxenbichler, and Wolff, 1969). The proposed mechanism is



The products formed by enzymatic hydrolysis depend on pH. At pH 3 to 4 glucobrassicin (3-indolylmethyl glucosinolate), isolated from *Brassica* species, is hydrolyzed to produce indolylacetonitrile (an organic nitrile), sulfur, sulfate, and glucose. At pH 7 the products of hydrolysis are 3-indolylmethyl isothiocyanate, sulfate, and glucose (Ahmed et al., 1972). The 3-indolylmethyl isothiocyanate is unstable and breaks down to 3-hydroxymethylindole and the thiocyanate ion.

Glucosinolates are mainly found in members of the Cruciferae family (in all 300 species examined) and consist, so far, of some 50 compounds of which only one or two predominate in any given species (Table 4.4). Highest concentrations of glucosinolates are found in seeds, but roots and leaves also contain measurable amounts. Concentrations in plant tissues vary with environmental factors and species variety. Measurement of thiocyanate production in the crucifers usually involves disruption of the cells and incubation, during which time the glucosinolates are hydrolyzed to thiocyanates. This amount is then referred to as the thiocyanate content of the tissue. For example, the thiocyanate content of Burpee White radish roots increased linearly with sulfate levels in plants grown in 0.5-strength Hoagland's solution, but the content did not increase in roots of the French Breakfast variety (Bible and Chong, 1975*a*). Neither variety showed an increase in thiocyanate content with sulfate when grown in double-strength Hoagland's solution. Thiocyanate content of Burpee White radish roots was greater on organic soil than on loam soil and increased with cooler conditions in both soils (Bible and Chong, 1975*b*). Spring- and fall-grown cruciferous vegetables often have a higher than average thiocyanate content. Thiocyanate content of Burpee White and Champion radish roots decreased as development

TABLE 4.4. GLUCOSINOLATES IN DOMESTICATED CRUCIFER PLANTS

Plant	Glucosinolate(s)[a] present
For food	
Brassica oleraceae cabbages, kale, brussel sprouts, cauliflower, broccoli, kohlrabi	Sinigrin Glucobrassicin Progoitrin Gluconapin Neoglucobrassicin
Brassica campestris turnips	Progoitrin Gluconasturtiin (*R*)-2-Hydroxy-4-pentenyl-glucosinolate
Brassica napus rutabaga	Progoitrin Glucobrassicin Neoglucobrassicin
Lepidium sativum garden crest	Glucotropaeolin
Raphanus sativus radish	4-Methylthio-3-butenyl-glucosinolate Glucobrassicin
For condiments	
Amoracia lapathifolia *A. rusticana* horseradish	Sinigrin Gluconasturtiin
Brassica carinata Ethiopian rapeseed	Sinigrin
B. juncea Indian or brown mustard	Sinigrin
B. nigra black mustard	Sinigrin
Sinapis alba white mustard	Sinigrin
Sinapis arvensis charlock	Sinigrin

(continued)

TABLE 4.4 (continued)

Plant	Glucosinolate(s)[a] present
For feed as processed seed meal	
Brassica campestris rape, turnip rape, Polish rape, rubsen, naverte	Gluconapin Progoitrin Glucobrassicanapin Glucoalyssin Glucoraphanin
Brassica napus rape, Argentine rape, winter rape	Progoitrin Gluconapin Glucobrassicanapin Gluconasturtiin Glucoiberin Sinalbin
Crambe abyssinica crambe, Abyssinian kale	*epi*-Progoitrin Sinigrin Gluconapin Gluconasturtiin

[a] Organic radicals in the glucosinolates given are as follows: sinigrin, allyl-; glucobrassicin, 3-indolymethyl-; progoitrin, (*R*)-2-hydroxy-3-butenyl-; gluconapin, 3-butenyl-; neoglucobrassicin, *N*-methoxy-3-indolymethyl-; gluconasturtiin, 2-phenylethyl-; glucotropaeolin, benzyl-; sinalbin, *p*-hydroxybenzyl-; glucobrassicanapin, 4-pentenyl-; glucoalyssin, 4-methylsulfinylbutyl-; glucoraphanin, 5-methylsulfinylpentyl-; glucoiberin, 3-methylsulfinylpropyl-; *epi*-progoitrin, (*S*)-2-hydroxy-3-butenyl-.

Source: Adapted from VanEtten, Daxenbichler, and Wolff, 1969, Table 1, p. 484. Data collected from several sources. Reprinted by permission of the publisher.

from seeding to the rosette stage occurred and remained at a low level during the reproductive stage (Chong and Bible, 1974). Thiocyanate content of foliage decreased during the rosette stage but increased considerably during the reproductive stage, especially the early bolting stage.

The goitrogenic effect of some members of the cabbage family (Brassicaceae) is apparently due to isothiocyanates (mustard oils), L-5-vinyl-2-thiooxazolidone (goitrin), and thiocyanate (Michajlovskij, Sedlák, and Kosterková, 1970). These compounds are released from glucosinolates when the plant material is crushed and the previously compartmentalized enzyme thioglucosidase (sometimes called by the trivial name myrosinase), which perhaps occurs in specific cells, is then able to hydrolyze the glucosinolates. The amount of these compounds varies considerably in different species. Raw cabbage and kale contained the equivalent of 10 to 30 mg isothiocyanate per 100 g tissue, kohlrabi contained 7 mg per 100 g, and cauliflower contained almost no isothiocyanates. Boiling vegetables alters the concentrations of the various constituents. Heating inactivates the enzymatic processes, but at higher temperatures thermal hydrolysis of glucosinolates and disintegration of goitrogens increases. In addition, the more volatile components are lost. Selection of vegetable varieties with lower glucosinolate concentrations is possible. For instance, rapeseed, cultivar Bronowski, has about one-twentieth the amount of unsaturated nitriles as the normal cultivar (*Brassica campestris*) and about one-tenth the amount of goitrin (Lo and Hill, 1972). In seeds of *B. campestris* var. Yellow Sarson, cyano-3,3-epithiobutane is the major product of glucosinolate hydrolysis, while in seeds of *B. campestris* var. Echo the major hydrolysis products are butenyl- and pentenylisothiocyanate (Kirk and MacDonald, 1974).

Glucosinolates are apparently biosynthesized from an amino acid; the nitrogen atom is retained in subsequent transformations (Underhill, Wetter, and Chisholm, 1973). Labeling experiments have demonstrated that L-methionine and D,L-cysteine are probable sources of the sulfur atom. Again, the function of these glucosinolates and their further metabolism within the cell are unknown. Conjecture is that glucobrassicin (3-indolylmethyl glucosinolate) may serve as an auxin reserve; however, conversions to auxin have not been unequivocally demonstrated (Miller, 1973). Although the formation of toxic thiocyanates and isothiocyanates from glucosinolates is difficult to experimentally test, it may serve as a protection mechanism for plants against foragers (Whittaker and Feeny, 1971).

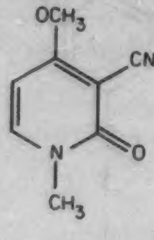
4.2.7 Indoleacetonitrile

Indoleacetonitrile (IAN), a substance which can be isolated from some plants, is structurally similar to indoleacetic acid, a naturally occurring auxin. Glucobrassicin, a glucosinolate found in many crucifers, can be converted to IAN by myrosinase (Section 4.2.6). Since this enzyme is normally separated from its substrate in the intact plant, IAN may be an artifact of the extraction methods used (Mahadevan and Stowe, 1972). This idea was further supported by the fact that IAN could be found only in plants containing glucobrassicin. However, some IAN was found in these plants even when precautions were taken to inactivate myrosinase. The limitations upon detection of minute quantities of metabolites must be considered before it is concluded that a given compound does not naturally occur.

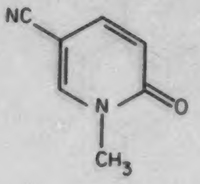
Indoleacetaldoxime (IAOX) can be converted to IAN and then to indoleacetic acid (IAA) in a variety of plants (Mahadevan and Stowe, 1972). The conversions of IAOX to IAN, to glucobrassicin, and to IAA in crucifers suggest that IAN may be a natural intermediate. The significance and relative importance of such a pathway for IAA formation in vivo is unknown. Although there is agreement that tryptophan is the precursor of IAA, the major pathway from tryptophan to IAA is uncertain. Schneider and Wightman (1974) discussed the evidence for severaal possible pathways of IAA biosynthesis.

4.2.8 Cyanopyridine Alkaloids

At present, the only cyanopyridine alkaloids which have been isolated from plant materials are ricinine,



isolated from *Ricinus communis* L. (castor bean), and nudiflorine,



isolated from *Trewia nudiflora* Linn. (Ferris, 1970). Biosynthesis of ricinine probably involves the incorporation of the nicotinamide skeleton into ricinine with subsequent formation of the nitrile group. If $H^{14}CN$ is fed to *R. communis*, the cyano group has a high specific activity. Since the amide group of asparagine also has a high activity, ricinine biosynthesis may proceed through either asparagine or β-cyanoalanine. The only data available for the catabolism of ricinine are for the bacterium *Pseudomonas*, which hydrolyzes ricinine to produce ammonia and an organic acid.

4.2.9 Nitrile Herbicides

The nitrile herbicides, which have been developed rather recently, presently consist of three major compounds: 2,6-dichlorobenzonitrile (dichlobenil), 4-hydroxy-3,5-diiodobenzonitrile (ioxynil), and 3,5-dibromo-4-hydroxybenzonitrile (bromoxynil) (Ashton and Crafts, 1973). Each compound is only sparingly soluble in water. Salts of ioxynil and bromoxynil are usually applied as sprays to foliage. Dichlobenil is often applied to soils to inhibit germination of seeds and growth of actively dividing meristems. It is volatile and is quickly lost from soils.

Metabolic studies of dichlobenil, usually with mature plants, have suggested the pathway outlined in Figure 4.3 (Verloop and Nimmo, 1969). The three- and four-position conjugated derivatives are as toxic as dichlobenil, while 2,6-dichlorobenzoic acid shows growth-regulating action. Since dichlobenil is generally used as a preemergence herbicide, metabolic studies with older plants may not directly relate to herbicidal properties in seedlings or embryos. Metabolism of ioxynil apparently leads to benzoic acid and iodide ions and "presumably bromoxynil would undergo similar reactions" (Ashton and Crafts, 1973). No data suggest that free HCN is formed by the metabolism of any of these nitrile herbicides. Dichlobenil

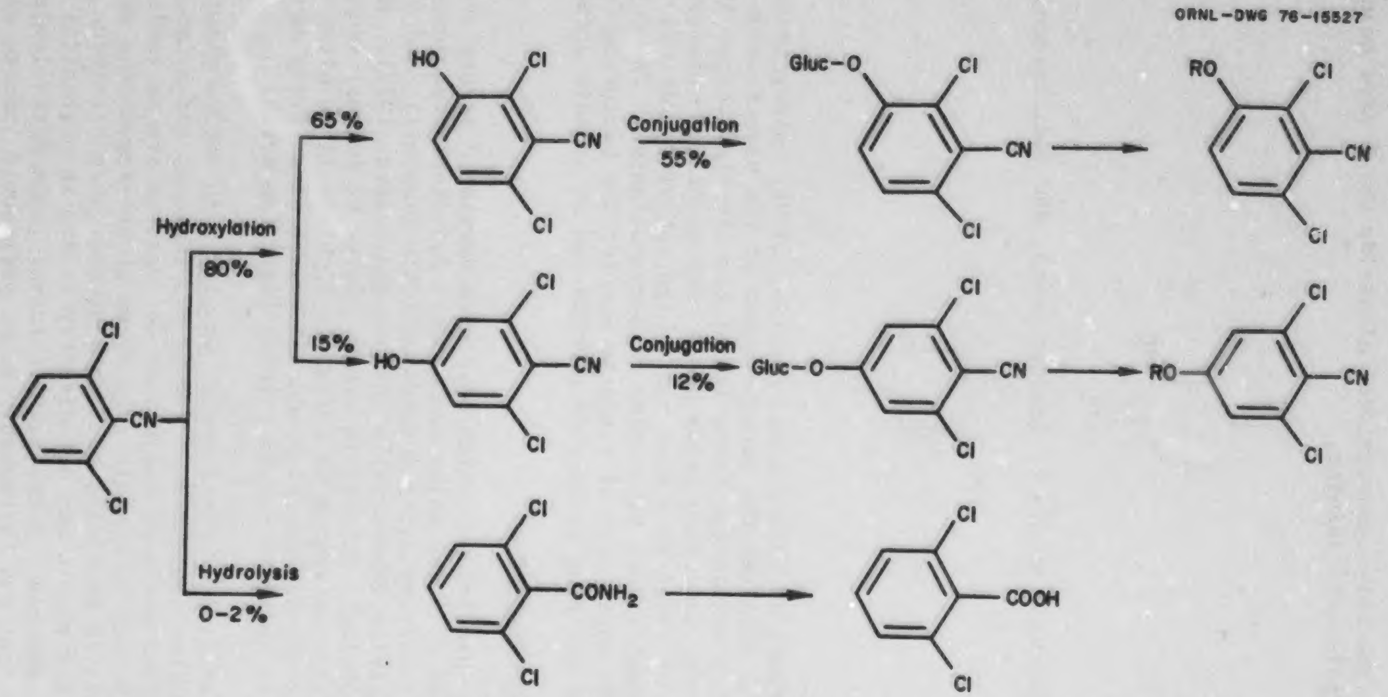

ORNL-DWG 76-15527
Cl
CN
Cl
Hydroxylation
80%
Hydrolysis
0-2%
65%
15%
HO
Cl
CN
Cl
Conjugation
55%
Conjugation
12%
Gluc-O
RO
CONH$_2$
COOH

Figure 4.3. Scheme showing the metabolites of dichlobenil in bean leaves after a five-day uptake of a 12 ppm solution via the roots (R = biopolymer, e.g., polysaccharides). Source: Verloop and Nimmo, 1969, Figure 2, p. 368. Reprinted by permission of the publisher.

can be absorbed by all plant organs, but movement through the plant is slow. One reason is that dichlobenil adsorbs to lignin, which is present in xylem walls. Little evidence exists for phloem transport. Some movement may occur by vapor diffusion through the intercellular air spaces in the plant.

Ioxynil can be translocated as evidenced by chlorosis on untreated leaves and by autoradiography after [^{14}C]ioxynil treatment (Ashton and Crafts, 1973). Although movement was slow, radioactive label was found in young leaves at the apex. Treatment of roots with [^{14}C]ioxynil resulted in the transport of small amounts to the foliage. Selective toxicity between grasses and broadleaf species and among different broadleaf species may partially be due to differential spray retention. Other factors must also operate, however, since selectivity could not be abolished by the use of wetting agents.

4.3 EFFECTS

4.3.1 Cyanide and Respiration

4.3.1.1 Inhibition of Respiration — The toxic effect of cyanide is due to its ability to inhibit various enzymes. Cytochrome oxidase, the terminal electron acceptor of the electron transport chain, is probably the most sensitive (Hewitt and Nicholas, 1963). Cyanide is known as a potent inhibitor of respiration and its action can ultimately lead to death of the organism. Although inhibition of cytochrome oxidase and other enzymes is usually due to complexing by cyanide of the metal ion from metalloenzymes, other inhibitory actions of cyanide are known (Section 4.3.3). Both undissociated cyanide (HCN) and cyanide ions (CN^-) inhibit cytochrome oxidase. It is uncertain whether cyanide binds with the Fe^{3+} or the Fe^{2+} form of cytochrome oxidase.

4.3.1.2 Cyanide-Insensitive Respiration — Although cyanide is a potent inhibitor of cytochrome oxidase, respiration, as measured by oxygen uptake, is not completely inhibited by cyanide in many plant species. The proportion of respiration that is insensitive to cyanide varies with the particular species and plant organ and with the physiological state of the tissue or organ (Henry and Nyns, 1975). Table 4.5 lists species that have cyanide-insensitive respiration (CIR) and the extent to which cyanide either inhibits or, in some cases, stimulates respiration.

Mitochondria appear to be the organelle in which the insensitive respiration occurs. Use of inhibitors and various substrates have established that a CIR path coexists with the main respiratory electron transport chain (ETC). Whether the CIR path branches from the main electron transport chain or whether it is an independent chain utilizing similar substrates is uncertain. Hydroxamic acids apparently can inhibit the CIR pathway but not the normal ETC pathway.

Some adenosine triphosphate (ATP) production does occur during operation of the CIR pathway. In most systems exhibiting CIR, the adenosine diphosphate (ADP) to oxygen ratio in the presence of cyanide is about 1 compared to about 3 for the ETC. Most of the CIR is also insensitive to anti-

TABLE 4.5. RELATIVE CYANIDE-INSENSITIVE RESPIRATION OF HIGHER PLANTS

Species	Relative respiration rate (%)[a]			
	Whole tissue	Cell-free extract	Isolated mitochondria	Submitochondrial particles
	Monocotyledoneae			
Arales				
Araceae				
Aerissina amurense	102			
Amorphophallus rivieri	124			
Arum creticum	96			
Arum italicum	100	100	95	
Arum maculatum	150		100	
	100		73	
	175		80	
			94	
			45	
			162	
Biarum tenuifolium	95			
Philodendron grandifolium	159			
Sauromatum guttatum	50		100	
Symplocarpus foetidus	123		82	
			62	
			75-100	
			50	
			124	
			97	
			86	
			61	
			62	
Liliaceae				
Allium cepa	87			
Graminales				
Gramineae				
Hordeum sp.	81			
	36			
Hordeum vulgare	70			
Orisa sp.	75			
Orisa sativa	50			
Triticum sp.	145			
	115			
	91			
Triticum aestivum	35			
Zea mays	147			
	Dicotylodoneae			
Laurales				
Lauraceae				
Persea americana	109			
Persea gratissima	132			
Leguminosales				
Leguminosae-Papilionoidea				
Lathyrus odorata	100			
Phaseolus aureus			20	20
			36	
			65	
			25	
Phaseolus vulgaris			20	
			34	
Phaseolus sp.	125			
Pisum sativum			15	
Vigna sinensis			20	
Fagales				
Fagaceae				
Fagus sylvatica	145			
	115			

(continued)

TABLE 4.5 (continued)

Species	Relative respiration rate (%)[a]			
	Whole tissue	Cell-free extract	Isolated mitochondria	Submitochondrial particles
Sapindales				
Aceraceae				
Acer pseudoplatanus			62	
Coniferales				
Cupressaceae				
Cedrus atlantica	102			
Cedrus libani	96			
Cruciferales				
Cruciferae				
Brassica napus	123			
Brassica oleracea			10	
Sinapsis alba	120			
Chenopodiales				
Chenopodiaceae				
Beta vulgaris				
red beet	111			
	131			
sugar beet	105			
Polemoniales				
Convolvulaceae				
Ipomea batatas	121		50	
			68	
			38	
			61	
			33	
			49	
Solanales				
Solanaceae				
Lycopersicon esculentum	58			
Nicotania tabacum	64			
Solanum tuberosum	110		30	
	75		30	
	94		58	
	89			
	58			
	84			
Ombellales				
Umbelliferae				
Daucus carota	106			
	151			
	128			
	30			
Asterales				
Compositae				
Helianthus tuberosus			35	

[a] 100 x the ratio of the respiration rate measured after the addition of cyanide to the respiration rate measured in the absence of cyanide. Specific cyanide concentrations were not included.

Source: Adapted from Henry and Nyns, 1975, Table 1, pp. 4-5. Data collected from several sources. Reprinted by permission of the publisher.

mycin, an inhibitor acting on the cytochrome *b* level. Since ATP production at sites II and III occurs after cytochrome *b* in the ETC, ATP is probably not produced at these sites during cyanide inhibition. Synthesis of ATP may occur at site I before cytochrome *b* if the cyanide-insensitive pathway branches off the ETC, or alternatively, the CIR chain may contain just one site of ATP synthesis.

An important facet of the CIR pathway is that it is inducible in certain organisms and is often related to ageing or changes in physiological state (Henry and Nyns, 1975). For example, fresh potato tuber discs had 70% of their respiration inhibited by 0.4 m*M* NaCN, but after ageing in water for 20 hr the entire endogenous respiration was insensitive to 0.4 m*M* NaCN. Infection of *Scorzonera* discs by *Agrobacterium tumifaciens* decreased the cyanide inhibition (using 1 m*M* NaCN) from 60% in uninfected discs to 25% in infected discs. Injury can also increase the percentage of respiration insensitive to cyanide. Higher concentrations of HCN (1 m*M*) decreased the difference between uninfected and infected tissue to 75% and 65% inhibition, respectively. Protein synthesis is apparently necessary for induction of the CIR chain although the exact roles of cytoplasmic and mitochondrial protein biosynthesis are not yet defined.

Flow of electrons through either the ETC or the CIR chain is in some way modulated in the cell. The physiological role of such a pathway is not completely understood. The high CIR in portions of the inflorescence in some araceous plants (e.g., skunk cabbage) produces an increase in temperature and may be related to flowering at the end of winter. The occurrence of the CIR pathway in injured tissues may serve to remove toxic or unwanted substances, especially if ATP synthesis "is not required or is impossible, as is the case in tightly coupled mitochondria that have depleted ADP" (Henry and Nyns, 1975).

4.3.2 Inhibition of Photosynthesis

Photosystem I is inhibited by KCN, presumably at the site of plastocyanin, a component of the photosystem electron transport chain. With isolated chloroplasts and purified spinach plastocyanin, Berg and Krogmann (1975) found evidence that KCN removes copper from plastocyanin. The apoprotein produced, apoplastocyanin, remains in the chloroplast membrane and is unable to transfer electrons to photosystem I.

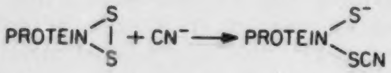
4.3.3 Inhibition of Enzymes

Besides the inhibition of cytochrome oxidase (Section 4.3.1.1), cyanide also inhibits a variety of other enzymes. Inhibition can occur (1) by the complexing of the cyanide ion with metals in metalloenzymes, (2) by the combination of undissociated HCN with carbonyl groups of aldehydes or ketones to form cyanohydrins, or (3) by the irreversible reaction of sodium cyanide with disulfide bonds (Hewitt and Nicholas, 1963).

Inhibition by reaction of cyanide with metals in enzymes has been demonstrated in several cases, including uricase, nitrate reductase, tyrosinase, carbonic anhydrase, and carboxypeptidase (Hewitt and Nicholas, 1963). Many complexities exist in the inhibition patterns observed for certain enzymes. In spinach, the NADH-dependent nitrate reductase is inhibited by cyanide only when the enzyme complex is in the reduced state (Relimpio et al., 1971). Preincubation of the enzyme with nitrate reduces or abolishes the cyanide inhibition. The authors suggested that molybdenum, an essential cofactor of the enzyme, in a reduced state is irreversibly complexed by cyanide. In many cases of enzyme inhibition by cyanide, the exact mechanism of inactivation is unknown. The mere presence of a metal within the functional enzyme does not mean that the observed inhibition by cyanide is through metal chelation. Studies with the use of other inhibitors which react with carbonyl and disulfide groups are necessary to determine the inhibitory mechanism.

Yeast phenylalanine ammonia-lyase is inactivated by NaCN, presumably by reaction with carbonyl groups (Hodgins, 1971). The active center of the enzyme contains a carbonyl group and a linear relationship was observed between enzyme inactivation and incorporation of $Na^{14}CN$ into the enzyme, suggesting that the labeling and inactivation events are the same. Hewitt and Nicholas (1963) also reported that cyanide might inhibit by reacting with a carbonyl-containing substrate for a given enzyme and suggested that this may be the manner of inhibition for amino acid decarboxylases which use pyridoxal phosphate as a coenzyme.

An example of the third inhibition mechanism is the inactivation of xanthine oxidase by cyanide (Massey and Edmondson, 1970). The addition of cyanide to disulfide bonds leads to the production of the thiocyanate group, possibly by the following reaction:



Additional data suggest that persulfide formation may also be involved with the following scheme at the active site of the enzyme:

$$\text{PROTEIN}-S-S^- + CN^- \longrightarrow \text{PROTEIN}-S^- + CNS^-$$

4.3.4 Physiological Effects

When cyanide is added, the main effect first observed is inhibition of respiration. This inhibition can ultimately result in death of the cell, tissue, organ, or organism, presumably because no ATP is formed to serve the many processes which require a continuous supply. Lack of ATP leads to breakdown of many cellular functions and ultimately leads to death.

Because the major site of inhibition is in electron transport, cyanide has been a useful tool for determining if various processes directly depend on aerobic respiration. For example, cyanide causes a rapid depolarization of the electrical potential of plant cell membranes (Anderson, Hendrix, and Higinbotham, 1974; Higinbotham, Graves, and Davis, 1970). Depolarization results from inhibition of an electrogenic pump (membrane-bound) apparently caused by a decreased ATP concentration in the cell. The ATP decrease and electropotential decay have similar half-times (Slayman, Lu, and Shane, 1970). However, care must be used in interpreting data obtained with the use of inhibitors. In this case, cyanide, an effective metal chelator, may also be acting on a metalloprotein at the site of the pump (Anderson, Hendrix, and Higinbotham, 1974).

There are few studies on the effects of low cyanide concentrations — concentrations less than that which inhibits respiration. This lack of reported data is probably due to the sensitivity of the cytochrome oxidase enzyme system in the plant and the effective metabolism of cyanide to asparagine in a variety of plants (Section 4.2.1). However, some interesting observations have been reported and are discussed in the following sections.

4.3.4.1 Germination — Several reports have demonstrated that cyanide exposure can increase or stimulate germination in several species. For example, Mullick and Chatterji (1967) found that seeds of two legumes, *Clitoria ternatea* Linn. and *Rhyncosia minima* D.C., showed increased inhibition, germination, and early seedling growth when exposed to cyanide solutions. The maximum effect was observed when the seeds were soaked for 24 hr in 100 ppm NaCN at 35°C. Roberts (1969) summarized the data supporting oxidation hypotheses which explain dormancy and the release from dormancy in cereals. Presumably, most respiratory metabolism during early germination is through the pentose phosphate pathway (PP). The terminal oxidase involved is cyanide insensitive and, hence, germination would not be inhibited by cyanide. A small amount of respiratory metabolism occurs by the Embden-Meyerhof-Parnas glycolysis pathway and, thus, by the citric acid cycle and the ETC; therefore, cyanide stimulation of germination could be explained by an increased inhibition of this pathway, with shunting of metabolites into other pathways, perhaps the PP pathway. The relative importance of the PP pathway decreases during the later stages of germination (to different extents in different species), and degrees of cyanide inhibition can be observed at this time. The terminal oxidase, which is cyanide insensitive and operates during the early stages of germination, has not been identified.

Hendricks and Taylorson (1972) found that potassium cyanide in concentrations from 0.003 to 3.0 m*M* (maximum at 0.1 m*M*) promoted seed germination in lettuce (*Lactuca sativa* L.) and pigweed (*Amaranthus albus* L.). They suggested that blocking of the ETC promoted germination and that germination, therefore, may involve cyanide-insensitive respiration. They supported this hypothesis with data showing that aryl hydroxamates and thiocyanates, both of which inhibited cyanide-insensitive respiration in some systems, also inhibited germination in *Amaranthus* and *Lactuca*. The relation of these observations to operation of the PP pathway was not explored. Whatever mechanism exists, the crucial observation is that cyanide inhibition of the ETC allows for an altered metabolism which, in an unknown manner, stimulates seed germination in different species.

4.3.4.2 Anomalous Growth Response in Presence of Iron — Israelstam (1968) reported that cyanide retarded growth of *Phaseolus vulgaris* more severely in the presence than in the absence of iron. Subsequent data showed that a similar phenomenon occurred in wheat seedlings (Israelstam, 1970), although in both wheat and bean, growth in the absence of cyanide was greater than in its presence. In nutrient solution experiments, iron did not prevent the uptake of cyanide ($K^{14}CN$) (Alam and Israelstam, 1975). The presence of cyanide in the nutrient solution slightly decreased the amount of chlorophyll in the leaves. Photosynthesis and respiration were inhibited by cyanide to about the same extent as height growth. Cyanide plus iron inhibited photosynthesis and respiration 63% and 70%, respectively, of iron-treated controls. Cyanide without iron inhibited photosynthesis and respiration 34% and 50%, respectively, of untreated controls. Similar kinetics for the appearance of ^{14}C in leaves were observed in the presence and absence of iron; the chemical form of ^{14}C in leaves was not determined. Thus, the extent of metabolism of ^{14}CN could not be estimated. While these observations are interesting, considerable work is necessary to determine their importance.

4.3.4.3 Cyanide and the Translocation Process — Cyanide has been used to examine the translocation of photosynthate by phloem. Like other physiological processes inhibited by cyanide, the mode of transport inhibition is probably by inhibition of ATP production (i.e., inhibition of cytochrome oxidase). Pertinent questions concern whether cyanide exerts its effect locally in the sieve tubes or is carried in the transpiration stream and exerts its effect at the loading (source) site and whether the inhibitory effect is reversible. Application of cyanide (gaseous and aqueous forms) to stolons of *Saxifraga sarmentosa* L. inhibited transport of ^{137}Cs and natural ^{14}C assimilates, but cyanide did not move to any great extent toward the daughter plant (Qureshi and Spanner, 1973). The effect apparently occurred locally within the sieve tube, not at the source or sink, and was completely reversible.

4.3.4.4 Chromosomal Aberrations — The effects of cyanide on the occurrence of chromosomal aberrations have been examined in conjunction with work defining the mechanism of chromosomal mutation caused by radiation. D'amato and Gustafsson (1947) observed that pretreatment of barley with 1×10^{-4} and 1×10^{-3} *M* KCN prior to x-irradiation increased the mutation frequency in barley. However, pretreatments of 1×10^{-2} *M* KCN produced a lower mutation frequency than in the water presoaked controls or dry seed series. Lilly and Thoday (1956) found that cyanide and oxygen together produced an inhibition of mitosis and a higher percentage of chromosomal aberration in *Vicia faba* roots than that produced by the absence of oxygen. Treatments of cyanide without oxygen and oxygen without cyanide produced no observable increase in aberration frequency. Mikaelsen (1954) reported that 10^{-3} to 5×10^{-4} *M* NaCN decreased the frequency of production of chromosome fragments and chromosome bridges during exposure to gamma irradiation (11% to 25% protection). Kihlman (1957) and Kihlman, Merz, and Swanson (1957) confirmed Lilly and Thoday's results but also showed that *Vicia faba* exposed to x rays and KCN had a higher frequency of chromosome aberrations when oxygen was absent. A threshold concentration of 5×10^{-5} *M* KCN produced this effect, while for aberrations produced by KCN plus oxygen in the absence of x rays about 10^{-4} *M* was necessary. The stimulus for this work was the old hypothesis that H_2O_2 was the agent responsible for chromosomal aberrations produced by ionizing radiation. The justification for cyanide use was its ability to inhibit cytochrome oxidase, peroxidase, and catalase and thus to allow the buildup of peroxides in the cell.

4.3.4.5 Effects of Nitrile Herbicides — Many organic nitriles apparently have herbicidal properties. The relationships between the cyano group and herbicidal activity and among breakdown products of the herbicide are not understood (Barnsley and Yates, 1962). Although the cyano group may be important for herbicidal activity, HCN is not released from these compounds and, thus, activity is due to the particular structural characteristics of the whole molecule. All three herbicides apparently act as inhibitors of electron flow or uncouplers in respiration and/or photosynthesis (Ashton and Crafts, 1973).

4.3.4.5.1 Dichlobenil — Dichlobenil (2,6-dichlorobenzonitrile) is an inhibitor of germination and growth of plant parts which contain actively dividing meristems (i.e., embryos, buds, and shoot and root meristems). Both monocotyledons and dicotyledons are affected. Dichlobenil is often used as a preemergence herbicide for annual weeds in orchards and vineyards (Ashton and Crafts, 1973). Because it produces little contact damage and does not migrate to any depth in soil, trees and established crops with deeper root systems are not affected.

Dichlobenil inhibits active transport and cell division and, thus, growth. At lethal concentrations it produces a blackened appearance of apical meristems (Ashton and Crafts, 1973). Although it has limited movement in the plant, root treatment with dichlobenil decreases movement of assimilates from leaves to buds, stems, and roots. Dichlobenil has been observed to uncouple oxidative phosphorylation in mitochondria.

4.3.4.5.2 Ioxynil and bromoxynil — Ioxynil is a postemergence contact herbicide used to control broadleaf plants that are frequent weeds in cereal and grass crops and are resistant to 2,4-D (Ashton and Crafts, 1973). Bromoxynil is used to control broadleaf plants that tolerate phenoxy herbicides. Both bromoxynil and ioxynil are applied when weeds are in their early growth stages. Necrosis is the first visible symptom after bromoxynil or ioxynil treatment. Eventually, chlorosis occurs in the surrounding areas and in untreated leaves.

Ioxynil inhibits both respiration and photosynthesis. Stimulation of O_2 evolution was found at concentrations of 5×10^{-8} to 5×10^{-7} *M*, while inhibition of O_2 evolution was found at 5×10^{-6} to 5×10^{-3} *M* (Paton and Smith, 1967). Thus, uncoupling may occur at the lower concentrations and blockage of electron flow may occur at higher concentrations. Low concentrations of ioxynil can also inhibit electron transport in photosynthesis, perhaps at the level of plastoquinone. Herbicidal activity could be explained by either or both modes of action.

4.3.4.6 HCN as a Fumigant — Hydrogen cyanide released from $Ca(CN)_2$ is used as a fumigant for insect control in stored grain, tobacco and vegetable plant beds, greenhouses, and soils. It is registered for use on almonds, dried beans, citrus, cocoa beans, grains, nuts, dried peanuts, and spices (Thomson, 1974). Details of the fumigation process are described in Lindgren and Vincent, 1962.

The use of HCN is presently restricted and tolerance limits have been set for a variety of vegetables (Section 7.7, Table 7.4). Waldron, Robb, and Sleesman (1970) showed that greenhouse tomato fruits did not build up residues of cyanide after fumigation and airing. Various factors, however, affect the penetration of the fumigant into the fruit or vegetable and its retention within the food (Sinclair and Lindgren, 1958).

Little useful information was found on the chemical form of cyanide in fumigated foods or on metabolic conversions in these foods. Lopez-Roman, Barkley, and Gunther (1971) reported that the water solubility of HCN limits its use on many vegetables and most fruits other than citrus because in contact with moist commodities it usually causes burning, discoloration, wilting, and flavor changes, which render the commodities unmarketable. These authors demonstrated that citrus fruits can sorb considerable amounts of HCN, as measured by the decrease in HCN concentration in the fumatorium. These results were compared to an empty fumatorium control to determine sorption onto the fumatorium walls. No determinations of the chemical form of cyanide in the fruit were made. Desorbtion of cyanide was small over a 90-min period. Lindgren and Vincent (1962) reported that much of the sorbed HCN will be released from food due to its high vapor pressure. However, at pH 7 and above, some conversion to metal salts will occur and these salts will be stable within the food.

Page and Lubatti (1948) demonstrated that fumigation of dried fruit with HCN converted fructose into the corresponding cyanohydrin. The proportion of cyanohydrin formed increased dramatically with moisture content of the dried fruit. Even 50 days after fumigation, the total cyanide content was quite high.

Cyanide used as a preservative for raw vegetables may inhibit some enzymes that affect flavor and quality. Lipoxygenase catalyzes peroxidation of lipids and is thought to be responsible for quality deterioration in vegetables (Flick, St. Angelo, and Ory, 1975). Lipoxygenase was inhibited by cyanide to a greater extent in homogenates of purple eggplants than in homogenates of white or green eggplants.

SECTION 4

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SECTION 5

BIOLOGICAL ASPECTS IN WILD AND DOMESTIC ANIMALS

5.1 SUMMARY

As a general respiratory poison, cyanide would be expected to be toxic to many organisms that depend on aerobic respiration for life. It is rapidly absorbed from the gastrointestinal and respiratory tracts and has behaved similarly in all organisms tested. However, threshold levels do vary widely with different species.

The major threat of cyanide poisoning to livestock is through the ingestion of plants containing cyanogenic glycosides. The effects can vary from acute to chronic intoxication.

Cyanide generally is toxic to fish at 0.1 ppm. Species variations, of course, do occur with regard to cyanide sensitivity. Although cyanide is toxic to birds, there is little recent information on the effects of cyanide in birds. Invertebrates are also poisoned by cyanide; however, the toxicity levels, in most cases, are not well defined.

5.2 MAMMALS

5.2.1 Metabolism

Metabolism of hydrogen cyanide and its various salts by mammals is discussed in Section 6.2. Absorption readily occurs through inhalation, ingestion, or skin contact. The greatest danger to wild or domestic animals is through either ingestion of plants with a high cyanogenic glycoside content or deliberate poisoning by man.

5.2.2 Toxic Effects

This section discusses cyanide poisoning in animals that have eaten forage with a high cyanogenic glycoside content. Microbes in the ruminal fluid of sheep can hydrolyze cyanogenic glycosides to release free hydrogen cyanide (HCN) (Coop and Blakely, 1949). The hydrolytic enzyme present in the plant will also release some cyanide during digestion, but this amount is small compared to that released by the rumen microbes. Free HCN is released when ruminal fluid from the cow is incubated with glycosides or trefoil plant leaves containing glycosides (Bansal and Seaney, 1970). HCN is released in ruminant and nonruminant animals' stomachs. As a result animals grazing on sorghums, Sudan grasses, corn, and other cyanogenic plants may be poisoned. These animals manifest initial excitability with generalized muscle tremor; polypnea and dyspnea follow. They may also salivate, lacrimate, defecate, and urinate. Ultimately, there is loss of righting reflex, pupillary dilatation, gasping, and convulsions. Death can occur quickly (Burrows and Way, 1977), depending on the dose administered.

In acute cyanide poisoning, the effects are usually obvious. The animal is in severe distress and either dies or rapidly recovers. Chronic poisoning, however, is more difficult to diagnose. The often subtle effects may result secondarily from complications which develop from long-term cyanide ingestion. Horses grazing on Sudan grass and hybrid sorghums in the southwestern United States developed a syndrome with clinical signs including posterior ataxia, urinary incontinence, cystitis, and myelomalacia of the lower spinal cord (Van Kampen, 1970). Offspring of mares that had eaten Sudan grass during early pregnancy developed musculoskeletal deformities. Sudan grass and hybrid sorghums are known to be cyanogenic plants, but the disease symptoms were similar to lathyrism, which occurs from the ingestion of lupine or related plants. Van Kampen (1970) suggested that sorghum may contain lathyrogenic compounds since it possesses the metabolic precursors (Section 4.2.5).

A secondary effect from ingesting cyanogenic glycosides in sorghum and Sudan grass may be induced sulfur deficiency, which results when a significant proportion of the sulfur ingested by an animal is used to detoxify the cyanide released from these plants. Wheeler, Hedges, and Till (1975) found that sheep grazing on sorghum and Sudan grass forage gained significantly more weight when they were given supplemental sulfur. Sheep with access to salt licks containing 8.5% sulfur (average daily sulfur intake 0.82 g/sheep) gained up to 88% more live-weight than control sheep with access to licks containing 0.1% sulfur (average daily sulfur intake 0.01 g/sheep). The authors suggested that a sulfur deficiency might be suspected if animals fail to gain weight when grazing on young sorghum species with high HCN content.

A thyroid dysfunction occurring in sheep feeding on star grass (*Cynodon plectostachyum*), a plant with a high cyanogenic glycoside and low iodine content, is similar to a condition reported in some human populations (Section 6.3.3.2). In this case, the sheep developed enlarged thyroid glands and gave birth to lambs which were either stillborn or died shortly after birth (Herrington, Elliott, and Brown, 1971). Star grass only produces hypothyroidism during the rainy season when the cyanogenic glycoside content is high (180 ppm as HCN). According to Herrington, Elliott, and Brown (1971), an iodine and mineral supplement can prevent the effect of the glycosides.

5.3 FISH

5.3.1 Metabolism

Few data were found on the metabolism of cyanide in fish. Uptake and absorption of cyanide are apparently rapid since lethal quantities of cyanide cause death within minutes. Detoxification mechanisms are not well described. Doudoroff (1976) cited data showing that thiosulfate administered in the water with cyanide reduced the toxicity of cyanide. Presumably, the thiosulfate would increase the detoxification rate of cyanide to thiocyanate.

5.3.2 Effects

Cyanide release to waters is responsible for several fish kills each year (U.S. Federal Water Pollution Control Administration, 1964-1969). Most cyanide kills are usually through accidental release of cyanides to the environment rather than inadequate treatment procedures (Section 7.5). For example, a newspaper reported a fish kill in Melton Hill Lake near Oak Ridge, Tennessee, on August 22, 1975, which was attributed to cyanide from a metal-plating industry (Anonymous, 1975). In addition to pollution from human activities, decomposing plant materials which naturally occur in surface waters may contribute to the cyanide content of the water. The extent of this contribution is not known, however. Because industrial effluents containing cyanide may find their way into waterways, much information has been collected on toxicity of various cyanides to aquatic organisms. Table 5.1 lists a wide range of toxic levels for different fish. Concentrations greater than 0.1 ppm are toxic in most cases. Figure 5.1 demonstrates that the median lethal concentration of sodium cyanide for five species of freshwater fish decreased only slightly with increasing exposure time (Cardwell et al., 1976). Goldfish were found to be less sensitive than the other four species.

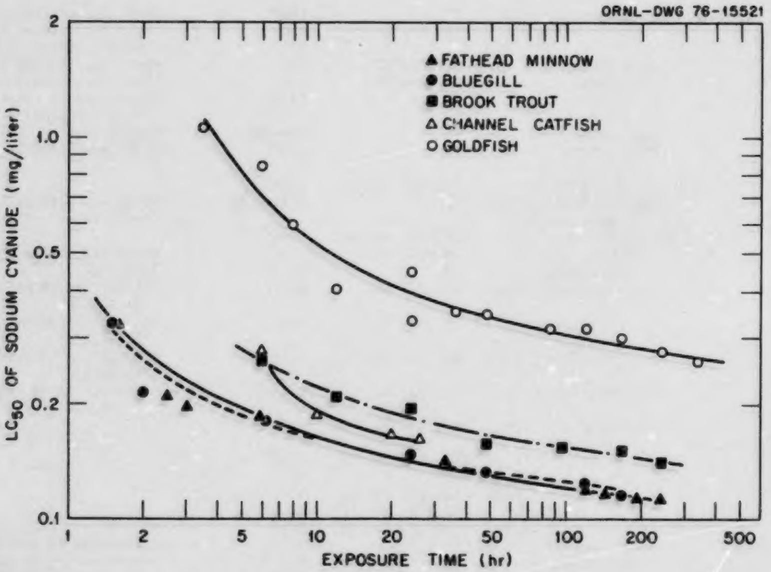

Figure 5.1. Relationship between median lethal concentration (LC_{50}) of sodium cyanide as CN^- and exposure time for five species of freshwater fish. Source: Adapted from Cardwell et al., 1976.

The toxicity of cyanides varies with temperature, dissolved oxygen, mineral concentration, and pH of the water (Doudoroff, 1976; National Academy of Sciences and National Academy of Engineering, 1972). The exact response differs among various species of fish.

TABLE 5.1. TOXICITY OF CYANURATES AND CYANIDES TO FISH

Chemical compound	Test organism	Test conditions[a]	Concentration (ppm)	Remarks
2-(2-Butoxyethoxy) ethyl thiocyanate	*Cyprinus carpio*	FW, LS	79, 93	No effect, 67 hr
	C. carpio	FW, LS	112	Death <60 hr[b]
1,3,3-Trimethyl-2-norbornyl thiocyanate and isobornyl thiocyanate	*C. carpio*	FW, LS	118, 131, 135	No effect, 43 hr[b]
Octyl thiocyanate	*C. carpio*	FW, LS	101, 104, 143	No effect, 48 hr[b]
2,4-Dinitrophenyl thiocyanate	*C. carpio*	FW, LS	140, 310	No effect, 91 hr[b]
Methanediol thiocyanate	*C. carpio*	FW, LS	74, 96, 104	No effect, 44 hr[b]
p-Dimethyl-aminophenyl thiocyanate	*C. carpio*	FW, LS	164, 174, 178	No effect, 46 hr[b]
Phenyl *p*-dimethyl-aminiso thiocyanate	*C. carpio*	FW, LS	129, 187, 202	No effect, 45 hr[b]
Allyl iso-thiocyanate	*Kuhlia sandvicensis*	SW, LS	0.05	Slight irritant response
	K. sandvicensis	SW, LS	0.1	Moderate irritant response
	K. sandvicensis	SW, LS	1.0	Violent irritant response
Ammonium thiocyanate	*Carassius auratus*	FW	1600	24-hr lethal limit
	Gambusia affinis	SB, FW, LS	910	24-hr TL_m, acute; high turbid water, 16–23°C
	G. affinis	SB, FW, LS	420	48-hr TL_m, turbid water
	G. affinis	SB, FW, LS	114	96-hr TL_m, turbid water
	K. sandvicensis	SW, LS	20.0	No observable response (2-min exposure)
	Lepomis sp.	FW	280–300	Killed in 1 hr
	Tinca tinca	FW	1600	24-hr lethal limit
Cyanide	*Lepomis auritus*	CB, SB, FW, LS	0.06[c]	
	Lepomis macrochirus	FW	0.12–0.18	96-hr TL_m, acute; hard and soft water
	K. sandvicensis			
	L. macrochirus	CB, SB, FW, LS	0.06[c]	
	L. macrochirus	SB, FW, LS	0.18	96-hr TL_m, acute
	L. macrochirus	FW	0.15	96-hr TL_m
	Lebistes reticulatus	CB, FW, LS	0.42	50% kill in 20 hr; pH 7.25; 24–24.5°C
	L. reticulatus	CB, FW, LS	0.28	50% kill in 30 hr; pH 7.25; 24–24.5°C
	L. reticulatus	CB, FW, LS	0.26	50% kill in 43 hr; pH 7.25; 24–24.5°C
	L. reticulatus	CB, FW, LS	0.24	<40% kill in 80 hr; pH 7.25; 24–24.5°C
	Notemigonus crysoleucas	CB, SB, FW, LS	0.06[c]	
	Micropterus salmoides	CB, SB, FW, LS	0.06[c]	
	Micropterus dolomieu	CB, SB, FW, LS	0.06[c]	
	Pomoxis annularis	CB, SB, FW, LS	0.06[c]	
	Pimephales promelas	CB, SB, FW, LS	0.06[c]	
	Salvelinus fontinalis	FW	0.1	Overturned in one day
		FW	0.2	Overturned in 50 min
		FW	0.08	Causes immediate signs of distress
	Trout	FW, FS, LS (river)	0.05	Will kill all fish in 130–136 hr
	Trout	FW, FS, LS	0.02	No kill in 27 days
	Trout	FW, FS, LS	1.0	Will kill in 20 min
	Fish[d]	FW, FS (river)	>0.1	Lethal to all fish
	Fish[d]	FW, FS (river)	0.05–0.1	Lethal to most game fish
	Fish[d]	FW, FS (river)	0.03	Lethal to some fish
	Fish[d]	FW	0.53–0.65	Toxic; toxicity increased over temperature range of 1.2 to 25.4°C

(continued)

TABLE 5.1 (continued)

Chemical compound	Test organism	Test conditions[a]	Concentration (ppm)	Remarks
Cyanide (CN^-)	*L. macrochirus*	SB, FW, LS	0.18	96-hr TL_m, acute
	L. macrochirus	SB, FW, LS	0.07	No kill in 96 hr
	L. macrochirus	SB, FW, LS	0.24	Total kill in 96 hr
Cyanide (a) zinc (b) (Zn^{++})	*L. macrochirus*	SB, FW, LS	0.26 (a)	96-hr TL_m, acute
	L. macrochirus	SB, FW, LS	3.90 (b)	
	L. macrochirus	SB, FW, LS	0.16 (a)	No kill in 96 hr
	L. macrochirus	SB, FW, LS	2.43 (b)	
Cyanides (metal complexes)	*L. macrochirus*	SB, FW, LS		Toxicity is generally a function of molecular HCN level.
n-Butyl carbitol thiocyanate	*K. sandvicensis*	SW, LS	20.0	Violent reaction, 2-min exposure
Hydrocyanic acid	*Lagodon rhomboides*	SB, SW, LS	0.069	24-hr TL_m, acute
	L. rhomboides	SB, SW, LS	0.10	All fish died
Hydrogen cyanide	Freshwater fish (cyprinids)	SB, FW, LS		Lowest lethal molar concentration was 7.7×10^{-6}.
	L. rhomboides	SB, SW, LS	0.069	24-hr TL_m, acute
	L. rhomboides	SB, SW, LS	0.05	Maximum dose, no kill
	Salmo gairdneri	SB, FW, LS	0.07	48-hr TL_m, acute; toxicity related to molecular HCN
Lauryl thiocyanate	*K. sandvicensis*	SW, LS	20.0	Violent reaction, 2-min exposure
Methyl isothiocyanate	*K. sandvicensis*	SW, LS	0.1	Slight irritant response, 2-min exposure
	K. sandvicensis	SW, LS	1.0	Violent irritant response, 2-min exposure
p-Chlorophenol isocyanate	*K. sandvicensis*	SW, LS	20.0	No observable response, 2-min exposure
Potassium cuprocyanide	*Rhinichthys atratulus*	CB, FW, LS	0.38	24-hr TL_m, acute; ≈20°C, pH 7.8–8.0; CN to Cu ratio of 4.0
	R. atratulus	CB, FW, LS	0.47	24-hr TL_m, acute; ≈20°C, pH 7.8–8.0; CN to Cu ratio of 3.7
	R. atratulus	CB, FW, LS	0.71	24-hr TL_m, acute; ≈20°C, pH 7.8–8.0; CN to Cu ratio of 3.0
	R. atratulus	CB, FW, LS	0.53	4-hr TL_m, acute; CN to Cu ratio of 4.0
	R. atratulus	CB, FW, LS	0.69	4-hr TL_m, acute; CN to Cu ratio of 3.7
	R. atratulus	CB, FW, LS	1.10	4-hr TL_m, acute; CN to Cu ratio of 3.0
Potassium cyanate	*K. sandvicensis*	SW, LS	20.0	No observable response, 2-min exposure
Potassium cyanide	*Brachydanio rerio*	SB, FW, LS	0.49	48-hr TL_m, acute; adults, 24°C, soft water
	B. rerio	SB, FW, LS	≈11.7	48-hr TL_m; eggs, 24°C, soft water
	C. auratus	SB, FW, LS	0.1–0.3	Killed in three to four days, hard water
	G. affinis	SB, FW, LS	1.6	48-hr TL_m, acute; high turbid water
	K. sandvicensis	SW, LS	0.1	Slight irritant response, 2-min exposure
	K. sandvicensis	SW, LS	1.0	Moderate irritant response, 2-min exposure
	K. sandvicensis	SW, LS	10.0	Violent irritant response, 2-min exposure
	L. macrochirus	CB, FW, LS	0.55	96-hr TL_m, acute; small fish
	L. macrochirus	CB, FW, LS	0.45	96-hr TL_m, acute; medium fish
	L. macrochirus	CB, FW, LS	0.57	96-hr TL_m, acute; large fish

(continued)

TABLE 5.1 (continued)

Chemical compound	Test organism	Test conditions[a]	Concentration (ppm)	Remarks
	S. trutta	CB, FW, LS	0.1	Threshold dose, 45 min; 15.6 C[h]
	S. trutta	CB, FW, LS	0.5	Threshold dose, 13 min; 15.6 C[h]
	S. trutta	CB, FW, LS	1.0	Threshold dose, 8.5 min; 15.6 C[h]
	S. fontinalis	SB, FW, LS	0.009	Reduced ability to swim by 50%; 8-10°C
	S. fontinalis	SB, FW, LS	≈0.09	48-hr TL_m, acute; 8-10°C
	S. fontinalis	SB, FW, LS	0.05-0.08	Minimum lethal concentration; 8-10°C
	Trout	FW	0.11	Loss of equilibrium in 2 hr; 7-9°C, dissolved oxygen 11 ppm
	Trout	FW	0.11	Loss of equilibrium in 10 min; 7-9°C, dissolved oxygen 3 ppm
	Fish[d]	FW, LS	0.25-0.35	Minimum lethal dose; hard water, 20°C
	Fish[d]	FW, LS	0.6-1.0	Minimum lethal dose; distilled water, 20°C
Potassium thiocyanate	*K. sandvicensis*	SW, LS	10.0	No observable response, 2-min exposure
	K. sandvicensis	SW, LS	20.0	Slight reaction; 2-min exposure
Sodium cyanide	*Anguilla anguilla*	CB, FW, LS	0.49	Killed in 12 hr; 17-18°C
	C. auratus	CB, FW, LS	4.9	Killed in 12 hr; 17-18°C
	C. auratus	SB, FW, LS	1.0	100% kill in 5-48 hr; 26°C, pH 7.2-8.7
	C. carpio	SB, FW, LS	1.0	100% kill in 5-14 hr; 26°C, pH 7.3-7.5
	Gasterosteus aculeatus	CB, FW, LS	0.49	Killed in 8 hr; 17-18°C
	Ictalurus melas	SB, FW, LS	0.25	Not lethal in 72 hr; 24.4°C
	Ictalurus natalis	SB, FW, LS	1.0	100% kill in 5-10 hr; 24-26° C, pH 7.0-8.2
	K. sandvicensis	SW, LS	2.0	Violent reaction, 2-min exposure
	K. sandvicensis	SW, LS	1.0	Slight reaction, 2-min exposure
	Lepisosteus osseus	SB, FW, LS	1.0	100% kill in 3.4-4.2 hr; 23°C, pH 7.3-7.8
	Lepomis cyanellus	SB, FW, LS	0.25	Lethal to most in 72 hr; 24.4°C
	L. cyanellus	SB, FW, LS	1.0	100% kill in 1.0-2.8 hr; pH 5.5-9.0
	L. cyanellus	SB, FW, LS	0.5	100% kill in 4.1-6.0 hr, 24-26°C, pH 7.6-8.0
	L. cyanellus	SB, FW, FS, LS	5.0	Moderately effective as repellant (lake study)
	L. cyanellus	SB, FW, FS, LS	1.0	Avoidance response
	L. cyanellus	SB, FW, FS, LS	<0.5	No response
	L. macrochirus	SB, FW, LS	0.15	96-hr TL_m, acute; hard water
	M. salmoides	SB, FW, LS	1.0	100% kill in 0.7-1.4 hr; 23-27°C, pH 7.0-8.9
	Minnows	FW, LS	0.3	No effect in 24 hr
	Minnows	FW, LS	0.5-0.7	25% mortality in 24 hr
	Minnows	FW, LS	0.8	100% mortality in 24 hr
	N. crysoleucas	SB, FW, LS	0.25	Not lethal in 72 hr; 24.4°C
	Phoxinus phoxinus	CB, FW, LS	0.49	Killed in 6 hr; 17-18°C
	P. promelas	SB, FW, LS	0.35	96-hr TL_m, acute; hard water, 25°C
	P. promelas	SB, FW, LS	0.23	96-hr TL_m, acute; soft water, 25°C
	P. promelas	SB, FW, LS	0.24	48-hr TL_m, acute
	S. trutta		0.49	Killed in 2 hr; 17-18°C
Sodium cyanide	*L. macrochirus*	SB, FW, LS	0.54 (a)	129 min, median resistance time
Total CN (a)	*L. macrochirus*	SB, FW, LS	0.50 (b)	
Molecular HCN (b)	*L. macrochirus*	SB, FW, LS	0.96 (a)	50 min, median resistance time
	L. macrochirus	SB, FW, LS	0.90 (b)	
	L. macrochirus	SB, FW, LS	0.72 (a)	91 min, median resistance time
	L. macrochirus	SB, FW, LS	0.62 (b)	

(continued)

TABLE 5.1 (continued)

Chemical compound	Test organism	Test conditions[a]	Concentration (ppm)	Remarks
	L. macrochirus	SB, FW, LS	0.16	48-hr TL_m, acute, adults; 24°C, soft water
	L. macrochirus	SB, FW, LS	5.0	Lethal in 1 hr
	L. macrochirus	SB, FW, LS	0.28	24- and 48-hr TL_m, acute; 20°C
	L. macrochirus	SB, FW, LS	0.62	96-hr TL_m; hard water, 18°C
	L. macrochirus	SB, FW, LS	0.35	96-hr TL_m; hard water, 30°C
	L. macrochirus	SB, FW, LS	0.45	96-hr TL_m; soft water, 18°C
	L. macrochirus	SB, FW, LS	0.33	96-hr TL_m; soft water, 30°C
	L. macrochirus	SB, FW, LS	0.47	48-hr TL_m; soft water, 18°C
	L. macrochirus	SB, FW, LS	0.33	48-hr TL_m; soft water, 30°C
	L. macrochirus	SB, FW, LS	0.44	48-hr TL_m; hard water, 18°C
	L. macrochirus	SB, FW, LS	0.39	48-hr TL_m; hard water, 30°C
	L. macrochirus	SB, FW, LS	0.45	96-hr TL_m, acute; toxicity is dependent on temperature, dissolved oxygen, and hardness of water
	L. macrochirus	SB, FW, LS	0.45	96-hr TL_m, acute; "normal" dissolved oxygen
	L. macrochirus	SB, FW, LS	0.12	96-hr TL_m, acute; "low" dissolved oxygen
	M. dolomieui	CB, FW, LS	0.1	Threshold dose, 200 min; 21.1°C[e]
	M. dolomieui	CB, FW, LS	0.2	Threshold dose, 30 min; 21.1°C[e]
	M. dolomieui	CB, FW, LS	1.0	Threshold dose, 14 min; 21.1°C[e]
	Rasbora heteromorpha	SB, FW	0.074	20% kill in seven days
	R. atratulus	CB, FW, LS	0.22	24-hr TL_m, acute; ≈20°C, pH 7.7-8.0
	R. atratulus	CB, FW, LS	0.26	4-hr TL_m
	S. gairdneri	CB, FW, LS	0.15	28.8-min mean survival time when acclimatized 24 hr in test tank; 50.8-min mean survival time when acclimatized 191 hr in test tank
	S. gairdneri	CB, FW, LS	0.16	39.0-min mean survival time of smallest fish (5.5-6.25 cm); 16.0-min mean survival time of largest fish (16.5-17.25 cm)
	S. gairdneri	CB, FW, LS	2.0	2.7-min mean survival time; 17-18°C, pH 7.4-8.0
	S. gairdneri	CB, FW, LS	0.3	8.8-min mean survival time; 17-18°C, pH 7.4-8.0
	S. gairdneri	CB, FW, LS	0.2	12.1-min mean survival time; 17-18°C, pH 7.4-8.0
	S. gairdneri	CB, FW, LS	0.1	2523-min mean survival time; 17-18°C, pH 7.4-8.0
	S. gairdneri	CB, FW, LS	0.105-0.155	Lethal; one-half died in 10 hr at <5 ppm; 17°C, pH 7.8-8.2; survival time increased at higher dissolved oxygen levels
	S. gairdneri	SB, FW, LS	5.0	Lethal in 1 hr
	S. gairdneri	CB, FW, LS	10.0	Total kill <3 min, acute; 17.5°C
	S. gairdneri	CB, FW, LS	0.31	Total kill in <10 min, acute
	S. gairdneri	CB, FW, LS	0.14	Total kill in 165 min, acute
	Salmo trutta	CB, FW, LS	0.2	62 min, mean death time; 15.6°C[f]
	S. trutta	CB, FW, LS	0.5	16 min, mean death time; 15.6°C[f]
	S. trutta	CB, FW, LS	1.0	8.5 min, mean death time; 15.6°C[f]
	S. trutta	CB, FW, LS	2.0	5 min, mean death time; 15.6°C[f]
	S. trutta	CB, FW, LS	0.1	Threshold dose, 300 min; 15.6°C[g]
	S. trutta	CB, FW, LS	0.5	Threshold dose, 30 min; 15.6°C[g]
	S. trutta	CB, FW, LS	1.0	Threshold dose, 12 min; 15.6°C[g]

(continued)

TABLE 5.1 (continued)

Chemical compound	Test organism	Test conditions[a]	Concentration (ppm)	Remarks
	L. macrochirus	SB, FW, LS	0.17 (a)	700 min, median resistance time
	L. macrochirus	SB, FW, LS	0.155 (b)	
Isobornyl thiocyanate	*K. sandvicensis*	SW, LS	1.0	Violent reaction, 2-min exposure
	K. sandvicensis	SW, LS	0.2	Slight reaction, 2-min exposure
Isobornyl thiocyanoacetate	*I. punctatus*	SB, FW, LS	1.5	24-hr TL_m, acute
	I. melas	SB, FW, LS	>1.5	24-hr TL_m, acute
	K. sandvicensis	SW, LS	0.1	No irritant response; 2-min exposure
	K. sandvicensis	SW, LS	1.0	Violent irritant response; 2-min exposure
	L. cyanellus	SB, FW, LS	0.6	24-hr TL_m, acute
	L. macrochirus	SB, FW, LS	0.4	24-hr TL_m, acute; data at 20-23 C, no increase in kill after 24 hr
	N. crysoleucas	SB, FW, LS	1.5	24-hr TL_m, acute
	S. gairdneri	SB, FW, LS	<0.7	24-hr TL_m, acute; 11°C
Thiocyanic acid (various esters)	*Ptychocheilus oregonensis*	SB, FW, LS	10.0	Acute death in 24 hr
	Oncorhynchus kisutch	SB, FW, LS	10.0	Acute death in 24 hr
	O. tshawytscha	SB, FW, LS	10.0	Acute death in 24 hr
	S. gairdneri	SB, FW, LS	10.0	Acute death in 24 hr

[a] SB = static bioassay; CB = constant-flow bioassay; FW = fresh water; SW = sea (salt) water; LS = lab study; FS = field study.

[b] Concentration is dose, milligrams per kilogram, force-fed; 18.3°C.

[c] No species survived more than 10 hr at doses over 0.06 ppm.

[d] Species not given.

[e] All data from graph; dissolved oxygen near saturation, ≈8.8 to 9.8 ppm.

[f] Data from graph; alkalinity and pH had little effect over ranges studied; dissolved oxygen 8.1 to 9.0 ppm.

[g] Data from graph; dissolved oxygen near saturation, ≈9.0 to 10.0 ppm.

[h] Data from graph; dissolved oxygen near 5.0 ppm.

Source: Adapted from Becker and Thatcher, 1973, Table J, pp. J.2-J.10. Data collected from several sources.

In a review of cyanide effects on fish, Doudoroff (1976) concluded that fish are more susceptible to cyanide at reduced oxygen concentrations. This susceptibility is more noticeable with lower levels of cyanide (about 0.1 ppm). If the dissolved oxygen concentration of the water is reduced from 10 ppm to 4 ppm, the toxicity of cyanide increases by a factor of about 1.4, possibly because the fish must pump more water through their gills to obtain enough oxygen (Skidmore, 1974). Morgan and Kühn (1974) measured the breathing rate of largemouth bass and found a rapid rate increase in response to a sublethal cyanide concentration.

Generally, an increase in water temperature increases the metabolic rate of fish, which, in turn, increases the toxicity of a respiratory poison such as cyanide (Doudoroff, 1976); however, there are some exceptions. Toxicity also varies with fish species (Table 5.1), age, stage in life cycle, activity, metabolism, and acclimatization (Doudoroff, 1976; Skidmore, 1974). Doudoroff has summarized the relationships among water temperature during exposure, acclimation temperature, and toxicity. Except for the above generality, no further simple relationships are apparent.

No general relationship between body size and resistance of a given fish species to cyanide is apparent (Doudoroff, 1976). For example, Anderson and Weber (1973) reported an excellent linear correlation between the lethal response to cyanide and the cyanide concentration in comparisons of groups of male guppies of similar weight. The linear correlation between cyanide concentration and response in fish of different weight classes was poor. Manipulation of the data, however, did produce a linear correlation between concentration and the logarithm of the cyanide concentration divided by a fish weight factor. Doudoroff cited other examples in which resistance to a given cyanide concentration was not related to fish size.

The response to a given concentration of cyanide can be modified by previous exposure (Doudoroff, 1976). Neil (1957, cited in Leduc, 1966) found that brook trout exposed to sublethal cyanide concentrations of 0.01, 0.03, or 0.05 mg/liter as CN^- survived longer than controls when they were subsequently exposed to KCN at 0.4 to 0.5 mg/liter as CN^-. When challenged at 0.3 mg/liter as CN^-, however, brook trout previously exposed to 0.03 or 0.05 mg/liter died sooner than controls. Trout previously exposed to 0.01 mg/liter lived longer than controls. Brockway (1966, cited in Leduc, 1966) obtained results with cichlids which were also difficult to interpret. Juvenile cichlids exposed to 0.02, 0.06, or 0.10 mg/liter cyanide as HCN were more resistant than controls to challenge with relatively low concentrations of cyanide. Those previously exposed to 0.1 ppm cyanide as HCN were less resistant than controls when challenged with relatively high lethal levels.

Natural waters which receive industrial wastes contain a mixture of pollutants and the contribution of each component to total toxicity is difficult to evaluate. Zinc and cyanide are reported to be antagonistic (Cairns and Scheier, 1968; Chen and Selleck, 1969). Marking and Dawson (1975) presented a method in an attempt to describe additive toxicity of chemicals in water and to assign significance to the additive toxicity index. Using the data of Cairns and Scheier (1968) on zinc and cyanide toxicity to fathead minnows, Marking and Dawson (1975) calculated an additive index of -1.37, which indicated considerable antagonism. This should be expected as the zinc ion forms a stable complex with cyanide.

Brown (1968) described another method to attempt to estimate the toxic potential of a mixture of poisons by dividing the concentration of each poison in a mixture by its individual 48-hr LC_{50} and summing these proportions. If the sum is less than one, less than half the fish would die in 48 hr and if greater than one, more than half would die in 48 hr. The values in Table 5.2 were determined for rainbow trout. In spite of obvious problems with this method such as unequal changes in toxicity of various components of the mixture with changing water quality (hardness, pH, alkalinity, dissolved oxygen, etc.), it is felt that it may serve as a guide but does not substitute for actual tests.

Doudoroff (1956, 1976) and Doudoroff, Leduc, and Schneider (1966), recognizing that industrial effluents often contain cyanide in association with heavy metals (e.g., electroplating and metal-finishing plant wastes), examined the effect of cyanide-heavy metal complex formation on toxicity to fish. The toxicity of cyanide complexes, of course, varies considerably. In most cases, the toxicity is less than that of a compar-

TABLE 5.2. ESTIMATION OF THE TOXIC PROPORTION OF EACH POISON AND THE TOTAL TOXICITY OF THE MIXTURE FOR RAINBOW TROUT

Poison	Concentration in water (mg/liter)	48-hr LC_{50} (mg/liter)	Proportion of 48-hr LC_{50}
Ammonia	6.3	31.13	0.20
Phenol	1.39	4.6	0.30
Zinc	0.62	2.8	0.22
Copper	0.12	0.4	0.30
Cyanide	0.01	0.07	0.17
Sum of the proportions			1.19

Source: Adapted from Brown, 1968, Table 2, p. 731. Reprinted by permission of the publisher.

able amount of free cyanide. It has been suggested that HCN alone often determines the toxicity of solutions of complex cyanides (Doudoroff, Leduc, and Schneider, 1966; Doudoroff, 1976). Because of the dissociation of the metallocyanide complex, free HCN would always be present in equilibrium with the complex. It would be difficult to dissociate the toxicity due to the metallocyanide complex itself from cyanide.

Formation of the nickelocyanide complex markedly reduces the toxicity of both cyanide and nickel. This complex is reasonably stable and is not decomposed by direct sunlight; however, dissociation occurs in dilute solution at an acid pH. For example, the toxicity to fish of the nickelocyanide complex (NaCN combined with $NiSO_4$) increased tenfold when the pH of the test solution was reduced from 7.8 to 7.5 (Doudoroff, 1956). A pH change from 8.0 to 6.5 increased the toxicity more than a thousandfold. The TL_{50} value was 0.75 mg complex per liter at pH 6.5 and 1300 mg complex per liter at pH 8. The basis for this observation is that a lowering of the pH value enhances the dissociation of the metallocyanide complex to form hydrogen cyanide.

Copper, which is fatal to fish at 1 ppm in soft water, can be detoxified by complexing with cyanide, as can the very toxic synergistic mixture of copper and zinc. In extremely soft water with thorough aeration, copper ions can be released from the cyanide complex and the solution becomes toxic.

One additional factor affecting the toxicity of a cyanide compound to fish is decomposition of wastes to HCN. Burdick and Lipschuetz (1970) reported a fish kill in a New York stream resulting from ferrocyanide and ferricyanide in concentrations well below the lethal concentration [up to 8732 ppm potassium ferrocyanide was nontoxic to trout in a 1-hr exposure (Ellis, 1937, cited in Burdick and Lipscheutz, 1970)]. This enhanced toxicity is attributed to the photodecomposition of these compounds liberating HCN (Section 2.2.8.2). Concentrations of potassium ferrocyanide or ferricyanide as low as 2 ppm were rapidly toxic to fish (*Rhinichthys atratulus atratulus*, *Semotilus atromaculatus atromaculatus*, and *Hybognathus regius*).

The literature on the toxicity of nitriles to fish has been reviewed by Doudoroff (1976). The toxicity of lactonitrile is similar to that of cyanide because it undergoes rapid hydrolysis in water to free cyanide. Median tolerance limits for acrylonitrile are in the 10 to 50 ppm range for several species of fish. The lethal effects of acrylonitrile may be attributed to factors other than the liberation of cyanide. Toxicity of other nitriles appears to be due predominantly to the organic nitrile itself rather than to cyanide.

5.4 BIRDS

5.4.1 Metabolism

There is a paucity of information on the uptake, absorption, and distribution of cyanide in birds. Also, there is little information concerning the excretion of cyanide or its metabolites by birds.

5.4.2 Effects

There is surprisingly little information on acute and chronic toxic effects of cyanide on birds. A bird kill, possibly due to ingestion of cyanogenic plant material, was reported in British Columbia (Cameron, 1972).

5.5 INVERTEBRATES

5.5.1 Metabolism

The details of cyanide uptake, absorption, transport, and biotransformation within invertebrates are not well known. Detoxification of cyanide in some groups of invertebrates may be similar to that found in mammals (National Academy of Sciences and National Academy of Engineering, 1972). Thiocyanates are formed in mollusks, parasitic helminthes, and insect larvae of *Gasterophilus equi* (Khan and Bederka, 1973). Rhodanese, the enzyme converting cyanide to thiocyanate, is also found in blowflies. There are not enough data, however, to generalize that all members of a given invertebrate taxa possess a similar detoxification mechanism.

Bond (1961*a*) has summarized information on relative susceptibility of different insects to HCN fumigation. The data suggested that more tolerant insects were able to exclude HCN from their bodies. Studies with the granary weevil, *Sitophilus granarius* (L.), showed uptake to be linear with time if the fumigant concentration remained constant (Bond, 1961*a*). Neither rhodanese activity (thiocyanate production) nor detoxification by combination with cysteine to form 2-imino-4-thiazolidine carboxylic acid was found. Similar results were obtained with the desert locust, *Schistocerca gregaria* Forsk. Later studies showed that HCN does inhibit respiration in insects, but complete inhibition was only observed in *S. granarius* (Bond, 1961*b*). The response of *S. granarius* was quickly inhibited (15 min) long before the lethal dose (4-hr LD_{50} is 8 mg/liter) was absorbed (4 hr). Isotopic tracer studies suggested that a major portion of the absorbed HCN is excreted as amino acids in the feces of *S. granarius* (Bond, 1961*c*). Some cyanide may also have been metabolized to cyanocobalamin and CO_2.

5.5.2 Effects

The effects of cyanide on invertebrates are not too well documented. "Paralysis" occurs in insects which have been fumigated with HCN; recovery occured in some organisms (e.g., *S. granarius*) which were exposed to LD_{50} concentrations of 8 mg/liter for 4 hr even though respiration was completely inhibited within 15 min (Bond 1961*a*, 1961*b*).

Defensive secretions of cyanide have been found in millipedes of the order Polydesmida (Eisner and Meinwald, 1966). These millipedes, when placed in HCN killing jars, also seem to be more tolerant of HCN. In the millipede *Apheloria*, cyanide is generated in a two-compartmented organ by hydrolysis of mandelonitrile. Cyanide generation occurs outside the gland when the components of the two compartments are mixed during ejection.

Table 5.3 lists the toxicity of cyanide to various aquatic invertebrates. The data are insufficient to determine sensitive or resistant groups of invertebrates. The National Academy of Sciences and National Academy of Engineering (1972) suggested that levels greater than or equal to 0.01 ppm cyanide are hazardous in the marine environment, whereas levels less than 0.005 ppm present minimal risk. They also suggested that a level of 0.005 ppm cyanide not be exceeded in any waters with aquatic life.

TABLE 5.3. TOXICITY OF CYANIDES AND THIOCYANATES TO AQUATIC INVERTEBRATES

Chemical compound	Organism	Test conditions[a]	Concentration	Remarks
Ammonium thiocyanate	*Asellus aquaticus* (amphipod)	FW	100 ppm	No noticeable harm
	Carinogammarus (amphipod)	FW	100 ppm	No noticeable harm
	Midge larvae	FW	50 ppm	Killed
Cyanides	*Cricotopus bicinctus* (midge fly)	FW, FS (river)	<3.2 ppm	Survived and matured
	Physa heteroclita (snail)	SB, FW, LS	0.432 ppm	96-hr TL_m, acute
Potassium cyanide	Bivalve larvae	SW	0.014 ppm	Lethal
	Daphnia magna (cladocera)	SB, FW, LS	2.0 ppm	24-hr and 48-hr TL_m, acute
	D. magna	SB, FW, LS	0.7 ppm	72-hr TL_m, acute
	D. magna	SB, FW, LS	0.4 ppm	96-hr TL_m, acute
	Hydropsyche sp., larvae (caddis fly)	SB, FW, LS	2.0 ppm	48-hr TL_m, acute; soft water, 20-22.2°C
	Lymnaeа sp., eggs (snail)	SB, FW, LS	796.0 ppm	24-hr and 48-hr TL_m
	Lymnaea sp., eggs	SB, FW, LS	147.0 ppm	72-hr TL_m
	Lymnaea sp., eggs	SB, FW, LS	130.0 ppm	96-hr TL_m
	Physa heterostropha	SB, FW, LS	1.08 ppm	96-hr TL_m, acute; "normal" dissolved oxygen
	P. heterostropha	SB, FW, LS	0.48 ppm	96-hr TL_m, acute; "low" dissolved oxygen
	Stenonema rubrum (mayfly)	SB, FW, LS	0.5 ppm	48-hr TL_m, acute
Sodium cyanide	*D. magna* (cladocera)	SB, FW, LS	<3.4 ppm	Concentration to nearly immobilize; Lake Erie water, 25°C
	Gammarus pulex (amphipod)	CB, FW, LS	0.005 *N*	Survive ≈1.5 hr; 13-14°C
	G. pulex	CB, FW, LS	0.0001 *N*	Survive ≈3 hr; 13-14°C; temperature and pH affect survival
	Polycelis nigra (planaria)	SB, FW, LS	0.0006 *M*	Toxic threshold, survives 48 hr; pH 6.8, 14-18°C

[a] SB = static bioassay; CB = constant-flow bioassay; FW = fresh water; SW = sea (salt) water; LS = lab study; FS = field study.

Source: Adapted from Becker and Thatcher, 1973, Table J, pp. J.2-J.10. Data collected from several sources.

SECTION 5

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SECTION 6

BIOLOGICAL ASPECTS IN HUMANS

6.1 SUMMARY

Cyanide, an extremely toxic substance, can quickly cause death after inhalation, ingestion, or cutaneous absorption. For example, a hydrogen cyanide concentration of 0.3 mg/liter (270 ppm) in air is immediately fatal to humans. Fortunately, recovery is common if treatment is instituted as quickly as possible. Humans may be exposed to cyanide as hydrogen cyanide, a cyanide salt, cyanogen, or other cyanide-containing compounds such as nitriles. Cyanogenic glycosides from some plants release cyanide upon hydrolysis, but symptoms are delayed following ingestion because hydrolysis is relatively slow.

A detectable amount of cyanide is generally found in all biological materials; however, cyanide usually is not cumulative. Therefore, plasma cyanide levels may not be significantly higher in normal persons than in individuals with greater than average exposure to cyanide (e.g., cigarette smokers). However, thiocyanate, the major detoxification product of cyanide, may be a better index of cyanide exposure, as it is present in significantly higher amounts in the plasma of cigarette smokers than in the plasma of nonsmokers.

Cyanide may be metabolized via several pathways. The major pathway is an enzymatic reaction (rhodanese) with thiosulfate to produce thiocyanate, which is excreted in the urine. Cyanide also may react with cystine to produce nontoxic 2-aminothiazoline-4-carboxylic acid, or it is believed to enter one-carbon metabolism through formic acid and be oxidized and exhaled as carbon dioxide. Reaction of cyanide to cyanate with hydroxocobalamin to form cyanobalamin also has been described. Cyanide is excreted in the urine predominantly as thiocyanate.

Cyanide is believed to produce its toxic effects predominately by combining with metal ions in enzymes. In acute poisoning, inhibition of cytochrome oxidase prevents body cells from utilizing oxygen resulting in a histotoxic anoxia.

Treatment of acute cyanide poisoning is based on both binding and detoxifying cyanide. Sodium nitrite is employed to convert hemoglobin to methemoglobin which binds cyanide as cyanmethemoglobin. Thiosulfate is given as substrate to convert cyanide to the less toxic thiocyanate in the presence of the enzyme rhodanese. Some cases of chronic cyanide exposure have been treated with vitamin B_{12a} (hydroxocobalamin).

Acute effects caused by cyanide depend on how fast and to what extent histotoxic hypoxia is produced. The quicker cyanide levels build up in tissues, the more severe the response. The first breath of cyanide at 2000 ppm causes immediate hyperpnea with collapse, convulsions, and cessation of breathing within one minute. When death occurs this rapidly, the total absorbed dose may be 7 mg/kg. Ingested cyanide salts are relatively more slowly absorbed and symptoms may not appear for 5 to 30 min. Up to 3.5 mg/kg body weight may be absorbed when death occurs.

Some nitriles release cyanide slowly and, initially, the entire molecule may exert a pharmacological action. With chronic intake of organic nitriles, such as the cyanogenic glycosides in many plant foodstuffs, the slow release of cyanide may result in chronic cyanide poisoning. Various tropical neuropathies, Leber's optic atrophy, and tobacco amblyopia are correlated with chronic cyanide uptake. Demyelination of nerves in the central nervous system as a result of cyanide-induced anoxia may be responsible for the neuropathies.

Experimental studies have shown that cyanide compounds can exert an anticancer effect. Laetrile therapy has been used experimentally as a cancer chemotherapeutic agent for some years, but its efficacy as an antitumor agent has been seriously questioned.

6.2 METABOLISM

6.2.1 Uptake and Absorption

6.2.1.1 Ingestion — Theoretically, ingestion of any compound containing the cyanide moiety could cause poisoning if the cyanide ion is released by digestive processes or intestinal microflora. For example, when potassium cyanide is rapidly absorbed through the gastric mucosa, victims have become unconscious in a matter of seconds and have died within minutes following ingestion of large cyanide doses (Gleason et al., 1969) or within an hour after a LD_{50} dose.

The percentage of a given dose absorbed is a factor of dose size and absorption rate: death may intervene before absorption is complete. Stomach content and release rate of the cyanide ion from the ingested compound are limiting factors for absorption. Although actual rates of cyanide absorption from the gastrointestinal tract are not available, epidemiological evidence leaves no doubt that cyanide can be absorbed faster than the body can normally detoxify or excrete it (Section 6.2.3), which allows a fatal level to be reached.

A major source of oral cyanide intake by humans is cyanogenic glycosides present in many plant foodstuffs (Sections 4.2.3 and 6.3.3.1). As opposed to the rapid appearance of symptoms following ingestion of hydrogen cyanide or a cyanide salt, appearance of symptoms is usually delayed several hours after ingestion of a cyanogenic glycoside such as that in bitter almonds or apricot kernels (Polson and Tattersall, 1969). The delay probably reflects the slower release of cyanide. Food in the stomach also tends to delay absorption and to moderate the lethality of a dose (Liebowitz and Schwartz, 1948). Experimentally, both dogs (Brocklehurst, 1934) and cattle (Couch, 1934) were protected from cyanide poisoning by carbohydrates in the stomach. In the studies using carbohydrates, the protective effect may be attributed to more than merely the general bulk effect of the food itself.

6.2.1.2 Inhalation — Hydrogen cyanide vapor is absorbed rapidly through the lungs (Gettler and St. George, 1934; Polson and Tattersall, 1969). Because HCN has a pKa of 9.2 and exists primarily as the acid under biological conditions, absorption across the alveolar membrane should be rapid (Fingl and Woodbury, 1970; Wolfsie and Shaffer, 1959). Human inhalation of 270 ppm HCN vapor brings death immediately, while 135 ppm is fatal after 30 min (Fassett, 1963).

Cyanide absorption following inhalation of very low concentrations is indicated by the observation that smokers have higher thiocyanate levels in plasma and other biological fluids than do nonsmokers (Maliszewski and Bass, 1955; Wilson and Matthews, 1966). Cyanide levels usually are not significantly different (Pettigrew and Fell, 1973; Wilson and Matthews, 1966), as these levels probably reflect rapid conversion of cyanide absorbed from inhaled tobacco smoke to thiocyanate (Johnstone and Plimmer, 1959; Osborne, Adamek, and Hobbs, 1956; Pettigrew and Fell, 1973). Inhalation of cyanide salt dusts is also dangerous because the cyanide will dissolve on contact with moist mucous membranes and be absorbed into the bloodstream (Davison, 1969; Knowles and Bain, 1968).

Inhalation exposure to cyanogen [(CN_2)] is likely because it is a gas at room temperature. Absorption values are not available, but the lethal dose for rats is greater than that of hydrogen cyanide (McNerney and Schrenk, 1960). Besides releasing hydrogen cyanide and cyanate upon hydrolysis, cyanogen is also an irritant causing eye and nasal inflammation at concentrations as low as 16 ppm.

Halogenated cyanogens such as cyanogen chloride or cyanogen bromide are comparable in toxicity to hydrogen cyanide when inhaled and cause marked irritation of the respiratory system with hemorrhage and pulmonary edema (Fassett, 1963; Prentiss, 1937). Quantitative absorption data are not available.

The organic nitriles are another class of potential cyanide-releasing compounds that may present an inhalation hazard. It is not possible to generalize on whether the primary toxicity of these compounds resides with the cyanide moiety, the organic nitrile molecule itself, or its metabolite(s). For example, the 2-cyanopyridinium ion is metabolized to liberate cyanide (Way and Way, 1968), whereas the 4-cyanopyridinium ion liberates no cyanide and is believed to be metabolized to a carboxamide. Although organic nitriles may have the same toxicity as HCN, this should not infer that the primary toxicity can be attributed to cyanide liberation.

6.2.1.3 Percutaneous Absorption — Hydrogen cyanide in either liquid or vapor form is absorbed through the skin (Drinker, 1932; Potter, 1950; Tovo, 1955; Walton and Witherspoon, 1926). Absorption is probably increased if the skin is cut, abraded, or moist. Many accidents involving skin contamination also involve inhalation exposure; the contribution due to skin absorption in these cases is difficult to assess. Potter (1950) described a case in which liquid HCN ran over the bare hand of a worker wearing a fresh air respirator. Cyanide inhalation was prevented, but the worker collapsed into deep unconsciousness within 5 min, suggesting significant percutaneous absorption.

Cyanogen vapor is apparently not absorbed percutaneously to a significant degree. Rabbits exposed to 10,000 ppm cyanogen for 8 hr under conditions preventing inhalation exposure exhibited no toxic effects (McNerney and Schrenk, 1960). Some organic nitriles are also absorbed through and irritate the skin (Dudley and Neal, 1942; Fassett, 1963; Graham, 1965).

6.2.2 Transport and Distribution

6.2.2.1 In Blood — Regardless of the route of uptake and absorption, cyanide enters the bloodstream and is carried to other body tissues. Cyanide readily forms complexes with metal ions such as iron or copper. This property is probably responsible for most of its acute toxic effects, particularly when cytochrome oxidase is involved (Sections 6.3.1 and 6.3.2). Within the bloodstream, cyanide can bind with methemoglobin to form nontoxic cyanmethemoglobin (Chen and Rose, 1952; Williams, 1959). As the plasma cyanide concentration decreases, cyanide continues to dissociate from cyanmethemoglobin and eventually is metabolized or excreted (Albaum, Tepperman, and Bodansky, 1946; Smith and Gosselin, 1966).

The site of major toxic effects, cytochrome oxidase, and the major detoxification system, rhodanese, are found almost exclusively intracellularly (de Duve et al., 1955). A minor conversion of thiocyanate back to cyanide can occur in red blood cells (Goldstein and Rieders, 1951, 1953).

Tobacco smoke is probably the most important daily source of cyanide exposure for the general population (1600 ppm HCN in cigarette smoke, reported in U.S. Department of Health, Education, and Welfare, 1964), but due to the rapid excretion and detoxification to thiocyanate, cyanide levels are not significantly higher in plasma of smokers than in plasma of nonsmokers (Wilson and Matthews, 1966). Plasma thiocyanate levels, however, are significantly higher in smokers than nonsmokers (Lawton, Sweeton, and Dudley, 1943; Maliszewski and Bass, 1955; Trasoff and Schneeberg, 1944; Wilson and Matthews, 1966) due to its slow excretion.

When sodium nitroprusside [$Na_2Fe(CN)_5NO$] is incubated with some biological materials, it releases cyanide. The most active biological material is red blood cells. Red cells are more active in rats and mice than in humans, but this difference disappears upon hemolysis of the cells, indicating a species difference in red cell permeability to nitroprusside (Hill, 1942; Page et al., 1955; Smith and Kruszyna, 1974). Cyanide has been reported to be released from nitroprusside via two nonenzymatic reactions: (1) a slow, nonspecific reaction with free sulfhydryl groups and (2) a fast reaction with hemoglobin. In animals, the free cyanide produced from nitroprusside has been reported to be from the reaction with hemoglobin (Smith and Kruszyna, 1974).

From a chemical viewpoint, cyanogen chloride (CNCl) should be relatively stable in blood since it decomposes slowly in aqueous solution up to pH 8 (blood pH is around 7.4); however, in reality CNCl is rapidly broken down in blood (Aldridge and Evans, 1946). Thirty percent or more of CNCl was converted to HCN in the presence of red blood cells; no HCN was produced with serum alone although the CNCl was still rapidly broken down. Aldridge and Evans (1946) concluded that the lethal effects of CNCl were due to the formation of HCN and that CNCl was eventually converted to thiocyanate, the major cyanide metabolite in the body (Section 6.2.3.1).

6.2.2.2 In Organs — When sufficiently sensitive detection methods were used, cyanide was found in trace amounts in all biological materials tested (Boxer and Rickards, 1951). The destruction of cyanide in blood and organs of female rabbits receiving HCN or KCN was reported by Ballantyne et al. (1972). Equal doses of the cyanide radical were administered (8 mg/kg in these studies). A comparison was made between cyanide levels in tissues containing blood and in those from animals perfused with saline to remove the blood (Table 6.1). Cyanide levels in whole blood and serum were higher after injection of HCN than after injection of KCN, reflecting

TABLE 6.1. COMPARISON OF CYANIDE CONCENTRATIONS IN TISSUES FROM RABBITS KILLED BY HCN WITH CONCENTRATIONS IN TISSUES FROM RABBITS KILLED WITH KCN

Tissue	Cyanide concentration mean ± standard error[a]		P^b
	HCN	KCN	
	Containing blood		
Skeletal muscle	35.0 ± 5.2	29.6 ± 2.4	<0.5
Kidney	74.7 ± 10.3	52.0 ± 11.0	<0.1
Liver	148.7 ± 32.3	82.0 ± 8.0	<0.1
Spinal cord	48.5 ± 4.9	36.8 ± 3.5	<0.1
Brain	145.3 ± 37.2	106.5 ± 12.4	<0.1
Whole blood	685.0 ± 83.0	453.0 ± 34.0	<0.05
Serum	275.0 ± 18.0	161.0 ± 21.0	<0.005
	Perfused with saline		
Skeletal muscle	9.3 ± 2.7	7.8 ± 2.4	<0.7
Kidney	11.0 ± 4.3	2.3 ± 1.1	<0.1
Liver	43.7 ± 13.5	6.5 ± 0.8	<0.025
Spinal cord	49.8 ± 14.7	22.5 ± 3.8	<0.2
Brain	289.0 ± 67.7	98.0 ± 5.0	<0.02
Whole blood	761.0 ± 129.0	438.0 ± 8.0	<0.05
Serum	261.0 ± 48.0	134.0 ± 8.0	<0.05

[a] Concentrations expressed in micrograms CN per 100 g wet tissue and micrograms CN per 100 ml blood or serum. Blood was removed from the left ventricle of perfused animals before saline was injected.

[b] Significance of difference in cyanide concentrations between animals killed with HCN and those killed with KCN.

Source: Adapted from Ballantyne et al., 1972, Table IV, p. 216. Reprinted by permission of the publisher.

a more rapid absorption of the weakly ionized HCN. The authors stated: "There was generally no significant difference in the concentration of cyanide in blood-containing tissues or saline-perfused tissues removed from animals killed with HCN compared with the concentration in corresponding tissues from rabbits killed with KCN; the only exception was a somewhat lower concentration of cyanide in perfused livers from the rabbits killed with KCN compared with perfused livers from the animals killed with HCN." However, it seems that the cyanide level in saline-perfused brain tissue is also significantly lower in KCN-killed animals than in HCN-killed animals.

Cyanide determinations of autopsy samples from three human cyanide poisonings were reported by Finck (1969) (Table 6.2). Two deaths resulted from ingestion of unknown amounts of a cyanide salt (which salt was not reported) and one death resulted from inhalation of cyanide gas.

TABLE 6.2. CYANIDE LEVELS IN HUMAN TISSUES AND FLUIDS AFTER FATAL CYANIDE POISONING

Sample	Cyanide content (mg/100 g or mg/100 cc)		
	Case 1[a]	Case 2[b]	Case 3[b]
Gastric contents	0.03	15	20
Lung		0.09	0.70
Blood	0.50	0.75	0.80
Liver	0.03	0.40	0.50
Kidney	0.11	0.35	0.40
Muscle		0.30	
Brain	0.07	0.25	0.06
Urine	0.20	0.20	
Fat		0.20	

[a] Death from inhalation of cyanide gas.
[b] Death from ingestion of cyanide salt.

Source: Adapted from Finck, 1969, Table 1, p. 357. Reprinted by permission of the publisher.

6.2.2.3 Placental Transfer — The relatively high pKa value of HCN and its ready absorption through the respiratory tract, gastrointestinal tract, and skin indicate that it also should cross the placenta; however, no direct data confirming placental transfer were found in the literature. Also, no fetal abnormalities were reported in women suffering from various neuropathies believed to be partly caused by chronic cyanide poisoning (Section 6.3.3.1). Andrews (1973) compared thiocyanate levels in maternal and cord blood samples from 50 consecutive deliveries and found a direct correlation ($r = 0.914$).

6.2.3 Detoxification

Although cyanide interacts with some substances in the bloodstream (Section 6.2.2.1), most reactions occur intracellularly within organs. These include detoxification reactions as well as biochemical reactions which have been attributed to producing the predominant toxic effects (Sections 6.3.2 and 6.3.3). Some of the possible metabolic pathways for cyanide are shown in Figure 6.1.

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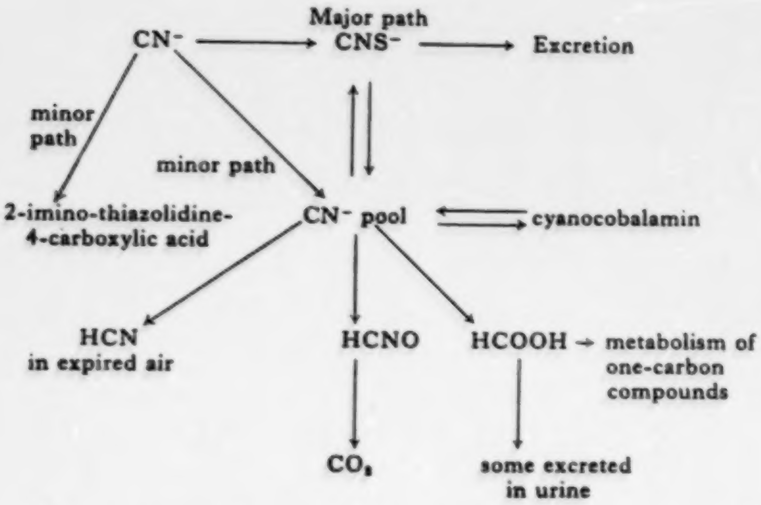
Major path
CN⁻
CNS⁻
Excretion
minor path
minor path
2-imino-thiazolidine-4-carboxylic acid
CN⁻ pool
cyanocobalamin
HCN in expired air
HCNO
HCOOH
metabolism of one-carbon compounds
CO₂
some excreted in urine

Figure 6.1. Fate of cyanide ion in the body. Source: Williams, 1959, p. 393. Reprinted by permission of the publisher.

6.2.3.1 Detoxification by Thiocyanate Production

— The conversion of cyanide to thiocyanate (SCN^-) is the major detoxification pathway, requiring a sulfur donor such as thiosulfate and the enzyme, rhodanese (Lang, 1933*a*, 1933*b*). Rhodanese (sulfurtransferase) is found to be widely distributed in animal tissues, especially in the liver. Cosby and Sumner (1945) partially purified this enzyme and it was subsequently crystallized by Sörbo (1953). Himwich and Saunders (1948) compared rhodanese activity in various tissues of dogs, rhesus monkeys, rabbits, and rats. They found that the rhodanese activity in liver, kidney, muscle, and adrenals was quite variable among different species, whereas brain rhodanese activity was similar among these species (Table 6.3). The dog differed from other species examined by having the highest enzyme activity in the adrenals rather than in the liver. Detoxification via rhodanese also occurs in other tissues; however the rhodanese contents usually are lower than the liver.

TABLE 6.3. RHODANESE ACTIVITY IN TISSUES OF THE DOG, RHESUS MONKEY, RABBIT, AND RAT
(mg CN converted to CNS per gram of tissue)

Tissue	Dog		Rhesus monkey		Rabbit		Rat	
	Range[a]	Number of observations	Range[a]	Number of observations	Range	Number of observations	Range	Number of observations
Suprarenals								
whole	2.14–3.60 (5.46, 4.50)	6	0.14–1.35	3	1.24–3.94	2	0.27–0.41	2
cortex	2.86–5.62	2						
medulla	0.27–1.12	2						
Liver	0.78–1.46 (4.91, 6.28)	7	10.98–15.16 (5.98)	4	7.98–18.92	9	14.24–28.38	9
Brain								
cortex	0.34–0.92	7	0.27	1	1.41–1.44	2	0.70–0.72	2
caudate nucleus	0.27–1.06	7	0.34–0.50	2	0.13–0.18	2		
midbrain	0.52–1.35	6	0.22–0.80	2	1.17–1.39	2	0.73–1.13	2
cerebellum	0.21–1.22	7	0.33	1	0.63–1.24	2		
medulla	0.38–1.52	7	0.49–0.85	2	0.91	1		
Spinal cord								
cervical	0.15–1.08	7	0.56–0.57	2	0.89–0.90	2	0.16–0.18	2
lumbar	0.12–0.84	4	0.20–0.42	2	0.35–1.74	2	0.23–0.27	2
sacral	0.16–1.41	4	0.23–0.28	2	0.59–1.10	3	0.56–0.74	2
Heart	0.11–0.14	6	0.48–0.82	3				
Kidney	0.42–0.74	6	2.46–3.58	4	6.20–7.69	3	10.44–11.08	2
Testes	0.32–0.41	5	0.38–0.46	3	0.32–0.36	2	1.24–1.61	2
Epidydymis	0.29	1						
Ovaries	0.42	1			0.30	1		
Lung	0.16–0.17	3	0.11–0.21	2	0.40	1		
Spleen	0.10–0.14	2	0.12–0.34	2	0.20	1		
Muscle	0.03–0.19	6	0.23–0.57	3	0.18	1		
Intestine								
duodenum	0.05–0.11	3						
jejunum	0.04	1						
Eye	0.02	1						
Optic nerve	0.35	1						
Salivary gland, parotid	0.05–0.36	3	0.99	1				
Lymph node	0.08–0.13	2						
Pancreas	0.14–0.28	4	0.12–0.44	2				
Thyroid	0.05–0.94	3						
Anterior pituitary	0.26	1						
Whole blood	0.01–0.02	2						
Erythrocytes	0.01–0.02	2						
Plasma	<0.01	1						

[a] Figures in parentheses are single observations falling outside the normal range.

Source: Adapted from Himwich and Saunders, 1948, Table 1, p. 351. Reprinted by permission of the publisher.

Sodium thiosulfate was found to be an effective sulfur donor for rhodanese (Table 6.4) (Chen and Rose, 1952; Himwich and Saunders, 1948). The substrate specificity of the sulfur donor for rhodanese was investigated by Sörbo (1953). These studies indicated that the substrate specificity for rhodanese requires that a sulfur atom be adjacent to another sulfur atom and that one of the sulfur atoms be free. Under optimal in vitro conditions, the rhodanese content in dog liver is sufficient to detoxify over 4000 g cyanide in 15 min. The comparable value for total skeletal muscle is 1763 g. These composite values are over 1000 times the lethal dose for dogs (Section 6.3.2). This infers that rhodanese itself, because of its high tissue content and turnover number, is not the limiting factor in cyanide detoxification. The rate limiting reaction probably can be attributed to the content and physiologic disposition of the sulfur donor. The thiosulfate content of the body is low and exogenously added thiosulfate can substantially increase the LD_{50} of cyanide. For example, Sheehy and Way (1968) increased the LD_{50} of potassium cyanide in mice by a factor of 4- to 6-fold by an intraperitoneal injection of sodium thiosulfate after signs of cyanide poisoning became apparent. Similar results were reported by Chen, Rose, and Clowes (1934), Frankenberg and Sörbo (1975), and others. Auriga and Koj (1975) found a protective effect of rhodanese and/or thiosulfate on the respiration of cyanide-poisoned, isolated mitochondria from rat liver and muscle and from beef liver and heart.

Rhodanese and cytochrome oxidase are localized in the mitochondria (de Duve et al., 1955); therefore, the rhodanese-catalyzed reaction must

TABLE 6.4. REPLACEMENT OF SODIUM THIOSULFATE BY OTHER SULFUR-CONTAINING COMPOUNDS

Compound	Percent activity of standard system
Sodium thiosulfate	100
Sodium sulfide	4
Sodium tetrathionate	Spontaneous conversion
Thiourea	4.5
α-Naphthylthiourea	4.6
Thiouracil	1
Dithiobiuret	1
Methionine	1
Cystine	1
Cysteine	1
Thiodiglycol	0
Diphenylsulfide	0
Diphenyldisulfide	0

Source: Himwich and Saunders, 1948, Table 3, p. 352. Reprinted by permission of the publisher.

occur intracellularly. The exogenous sodium thiosulfate has limited membrane permeability, especially when compared to cyanide (Crompton et al., 1974, cited in Auriga and Koj, 1975; Himwich and Saunders, 1948). Thiocyanate can be converted, to a limited extent, to cyanide; however, this dose not occur via rhodanese. There also is a minor conversion of organic thiocyanates to cyanide which is catalyzed by glutathione S-transferases (Habig, Keen, and Jakoby, 1975). These enzymes cleave organic thiocyanates to form cyanide and the respective asymmetric disulfide of gluthathione.

6.2.3.2 Minor Detoxification Pathways — Theoretically, any of the metabolic processes that convert cyanide into a less toxic compound or any substances that bind cyanide, such as methemoglobin, should reduce the toxicity of cyanide. Vitamin B_{12a} (hydroxocobalamin) occurs naturally in the body in small amounts (Drouet et al., 1951; Mollin and Ross, 1953); however, even if all the vitamin B_{12} in the liver was vitamin B_{12a}, it would combine with only 25 μg cyanide (Wokes and Picard, 1955). Hydroxocobalamin has a hydroxyl group bound to the cobalt atom and when cyanide is added to hydroxocobalamin, the cyanide is incorporated into vitamin B_{12} (cyanocobalamin) (Brink, Kuehl, and Folkers, 1950; Kaczka et al., 1950).

Formation of cyanocobalamin ($CN\text{-}B_{12}$), a cyanide-containing form of vitamin B_{12}, is important for two reasons: it is a minor cyanide detoxification pathway and it may minimize chronic cyanide intoxication (Section 6.3.3.1). Little or no cyanocobalamin is found in the plasma of normal human subjects (Linnell, MacKenzie, and Matthews, 1969). The major plasma B_{12} components are methylcobalamin (coenzyme B_{12}) and a mixture of hydroxocobalamin ($OH\text{-}B_{12}$) and deoxyadenosylcobalamin. Mushett et al. (1952) administered potassium cyanide to mice and successfully antagonized the toxic effect of cyanide with $OH\text{-}B_{12}$. The largest portion of cyanide in the urine appeared as $CN\text{-}B_{12}$ with a smaller portion as thiocyanate. There was no $CN\text{-}B_{12}$ detected in the urine of controls. The formation of $CN\text{-}B_{12}$ from other vitamin B_{12} chemical species may serve as a detoxification function by scavenging some cyanide which might otherwise react with cytochrome oxidase. Formation and excretion of $CN\text{-}B_{12}$ could deplete the body's store of active vitamin B_{12} during chronic cyanide exposure. The role of vitamin B_{12} in metabolic processes has been discussed by Brown (1973), Stadtman (1971), and Wokes and Picard (1955).

Vitamin B_{12} (cyanocobalamin) administered intraperitoneally or intravenously into mice produced no toxic effects, indicating that the cyano group is tightly bound (Winter and Mushètt, 1950), as this dose of vitamin B_{12} possessed a cyanide content of 32 mg/kg. In humans, half of an intramuscular or subcutaneous injection of vitamin B_{12} was excreted within 3 to 5 hr and almost all was excreted within 24 hr (Boxer and Rickards, 1951). The cyanide apparently was not released from the injected vitamin B_{12} in their experiments. Reizenstein (1967) reported that a therapeutic dose of cyanocobalamin in humans was excreted as cyanocobalamin in 12 hr in the urine. Guinea pig liver could convert cyanocobalamin to hydroxocobalamin in vivo and in vitro at a rate of 0.1 to 0.4 mμg/g of liver per day. Cima, Levorato, and Mantovan (1967) reported that cyanocobalamin was decyanated to hydroxocobalamin in vitro by rat liver and kidney. The decyanation was due to an enzyme system which they called "cyanocobalamin-decyanase." Release of cyanide from vitamin B_{12} in humans is supported by the observation that some patients treated for Leber's disease (Section 6.3.3.1) with vitamin B_{12} developed unusually severe optic atrophy, a condition associated with chronic cyanide poisoning (Foulds et al., 1968).

Hydroxocobalamin (100 to 250 mg/kg) is an effective cyanide antagonist. Hydroxocobalamin, but not cyanocobalamin, was effective in antagonizing cyanide intoxication and lethality in mice (Mushett et al., 1952). The studies on hydroxocobalamin have been confirmed by Friedberg, Grutzmacher, and Lendle (1965). Treatment of chronic cyanide poisoning in humans with hydroxocobalamin is discussed in Section 6.3.3.1. Additional information on the chemistry of vitamin B_{12} can be found in Brown (1973).

Although cyanide blocks the tissue utilization of oxygen (Section 6.3.1), therapy with oxygen nevertheless enhances the protective effects of other conventional cyanide antidotes (Burrows, Liu, and Way, 1973; Isom and Way, 1974; Sheehy and Way, 1968; Way, Gibbon, and Sheehy, 1966*a*, 1966*b*; Way et al., 1972).

Another minor detoxification reaction of cyanide occurring in vivo is the spontaneous reaction with cystine, yielding β-thiocyanoalanine which tautomerizes to 2-aminothiazoline-4-carboxylic acid or the equivalent isomer, 2-iminothiazolidine-4-carboxylic acid (Schöberl and Hamm, 1948). These products apparently are metabolically inert. Rats given subcutaneous injections of NaCN excreted 15% of the dose as 2-iminothiazolidine-4-carboxylic acid (Wood and Cooley, 1956).

The carbon atom of cyanide apparently enters the one-carbon metabolism through formic acid (HCOOH) and also is further metabolized and excreted as respiratory carbon dioxide (Boxer and Rickards, 1952*b*). These studies, using rats and dogs, were conducted with isotopically labeled $Na^{14}CN$ or thiocyanate ($NaS^{14}CN$). About 30% of the injected thiocyanate was oxidized to carbon dioxide in the rat during the nine-day experiment.

6.2.4 Excretion

A small amount of cyanide is eliminated unchanged through the lungs. Friedberg and Schwarzkopf (1969) found 1% to 2% of HCN given intravenously to guinea pigs was eliminated by the lungs before respiration ceased; this value was increased three- to fourfold when artificial respiration was applied. Hydrogen cyanide is found in respired air of normal humans and rats (Boxer and Rickards, 1952*a*). Most of the cyanide, under normal conditions, is metabolized to other compounds and excreted in the urine. The major metabolic product is thiocyanate, as discussed in Section 6.2.3.1 and shown in Figure 6.1. Thiocyanate is normally found in body fluids because cyanide and thiocyanate are related compounds and are regularly ingested in the diet (Section 6.2.1.1) and inhaled from tobacco smoke. Because tobacco smoke is a significant source of cyanide, thiocyanate levels in urine and other body fluids are higher in smokers than in nonsmokers (Lawton, Sweeney, and Dudley, 1943; Maliszewski and Bass, 1955; Pick, 1910; Wokes and Moore, 1958). Djuric, Raicevic, and Konstantinovic (1962) reported that thiocyanate appeared only sporadically and in trace amounts in the urine of nonsmokers. Thiocyanate levels in urine were directly proportional to the number of cigarettes smoked, varying from an average of 3.7 mg/liter with ten cigarettes per day to an average of 17.5 mg/liter with 40 cigarettes per day. Boxer and Rickards (1952*a*) found thiocyanate levels ranging from 8.3 to 20.0 mg/liter in urine from "normal subjects"; however, smoking histories were not given.

Wood and Cooley (1956) reported that in rats receiving NaCN, 80% of the cyanide was excreted in the urine as thiocyanate. Cyanide is also found in the urine but in much lower concentrations than thiocyanate (Boxer and Rickards, 1951). As mentioned in Section 6.2.3.2, cyanide is also excreted in the urine as cyanocobalamin.

6.3 EFFECTS

6.3.1 Mechanism of Action

Cyanide has a high affinity for certain metal ions. As a result, enzyme systems which require metal ions may be susceptible to cyanide inhibition. Oxidative enzymes and coenzymes in which iron is sometimes in the ferric (Fe^{3+}) state are especially sensitive. Cytochrome oxidase, the terminal enzyme in the mitochondrial electron transport chain is especially sensitive to cyanide and is completely inhibited by 3.3×10^{-8} moles/ml of cyanide, producing a cytotoxic anoxia which leads to the symptoms seen in acute cyanide poisoning (Chen and Rose, 1952; DiPalma, 1971; Way, Gibbon, and Sheehy, 1966*a*, 1966*b*). One molecule of cyanide combines with the iron in one molecule of cytochrome oxidase to form an inactive complex (Yoshikawa and Orii, 1972). Cyanide produces a histotoxic anoxia, that is, it prevents the tissue utilization of oxygen. Death from cyanide poisoning is probably due to a cerebral anoxia (DiPalma, 1971).

Cyanide combines with other heme-containing enzymes such as catalase and peroxidase as well as some nonheme enzymes such as tyrosinase, ascorbic acid oxidase, and phosphatase. However, these enzymes are much less sensitive to cyanide than cytochrome oxidase (DiPalma, 1971).

6.3.2 Acute Effects

In general, the effects of cyanide poisoning depend on the severity and rate of production of the histotoxic hypoxia. The quicker critical cyanide concentrations are attained in tissues, the more severe the effects and the smaller the dose required for a given effect. Inhalation of HCN leads to the most rapid absorption in tissues. Cyanide salts and other cyanide-containing compounds, such as organic nitriles and cyanogenic glycosides, release cyanide at different rates and in different amounts; therefore, the doses required for a given effect vary greatly. In addition to those effects due to cyanide, some cyanide-containing compounds have pharmacological effects not related to cyanide.

The acute effects produced by cyanide have been known for hundreds of years and many case studies of cyanide intoxication have been published. The course of events following exposure to cyanide has been described in numerous publications (DiPalma, 1971; Dreisbach, 1971; Fairhall, 1969; Gleason et al., 1969; Grant, 1974; McAdam and Schaeffer, 1965; Montgomery, 1965; Mooney and Quinn, 1965; Morgan and Seaton, 1975; Oke, 1969; Polson and Tattersall, 1969; Rentoul and Smith, 1973; Sollmann, 1957; Swinyard, 1970; Way and Way, 1968). The following general symptoms were compiled from these references.

Normally, a fatal cyanide exposure produces a brief stage of central nervous system stimulation followed by depression, hypoxic convulsions, and death. A concentration of 2000 ppm HCN in air (2.4 mg HCN per liter of air) gives a very brief sensation of dryness and burning in the throat, a feeling of warmth, and shortness of breath. The first breath produces immediate hyperpnea (deep, rapid breathing) and sometimes an outcry. Collapse, convulsions, and apnea (cessation of breathing) occur in less than a minute. The heart may continue to beat for several minutes after breathing stops. The dose causing rapid death may be less than 7 mg/kg body weight, while the LD_{50} for HCN absorbed through the skin (which takes much longer) is about 100 mg/kg body weight.

Similar effects follow ingestion of KCN or NaCN, but they may be slower due to the slower absorption from the gastrointestinal tract. Within 5 min hyperpnea occurs from chemoreceptor stimulation and vomiting results from irritation of gastric mucosa and central stimulation. Within 5 to 20 min after ingestion, a variety of symptoms are seen including unconsciousness; convulsions; flushed, hot, dry skin; full, rapid, irregular pulse; high systolic with low diastolic blood pressure; trismus of jaw muscles; and gasping. Hypoxic dilation of the pupils, vascular collapse, and cyanosis then occur.

Certain organic nitriles cause a sequence of events, such as CNS depression that may be due to the pharmacologic action of the entire molecule rather than cyanide per se. These effects may or may not be followed by cyanide poisoning, depending on whether cyanide is released. The slow increase in cyanide levels over a prolonged time may reveal symptoms which are due to inhibition of enzymes other than cytochrome oxidase. Tables 6.5, 6.6, 6.7, and 6.8 present quantitative data on the effects of various compounds which contain the cyanide moiety in humans and experimental animals.

6.3.3 Chronic Effects

The acute effects of cyanide on the body are well described and well recognized. However, the possible dangers of long-term exposure to low cyanide levels that in single doses do not produce clinical signs of poisoning are not well understood. Only recently have possible correlations been implicated between chronic cyanide uptake and specific diseases such as tobacco amblyopia, retrobulbar neuritis in pernicious anemia, Leber's optic atrophy, and Nigerian nutritional neuropathy.

TABLE 6.5. HUMAN RESPONSE TO INHALED CYANIDE AND CYANIDE-CONTAINING COMPOUNDS

Compound	Cyanide concentration		Response	Reference
	(mg/liter)	(ppm)		
Hydrogen cyanide	0.3	270	Immediately fatal	Prentiss, 1937
	0.2	181	Fatal after 10-min exposure	Prentiss, 1937
	0.15	135	Fatal after 30-min exposure	Prentiss, 1937
	0.12-0.15	110-135	Fatal after 1/2 to 1 hr or later, or dangerous to life	Fassett, 1963
Cyanogen		16	Nasal and eye irritation after 6 to 8 min	McNerney and Schrenk, 1960
Cyanogen chloride	0.40	159	Fatal after 10-min exposure	Prentiss, 1937
	0.120	48	Fatal after 30-min exposure	Fassett, 1963
	0.005	2	Intolerable concentration, 10-min exposure	Fassett, 1963
	0.0025	1	Lowest irritant concentration, 10-min exposure	Fassett, 1963
Cyanogen bromide	0.40	92	Fatal after 10-min exposure	Prentiss, 1937
	0.035	8	Intolerable concentration	Prentiss, 1937
	0.006	1.4	Greatly irritating to conjunctiva and the mucous membranes of the respiratory system	Prentiss, 1937

The various neuropathies probably result from demyelination of nerves in the central nervous system brought about by cyanide-induced anoxia. Ferraro (1933) gave repeated doses of cyanide to cats and monkeys (amount not reported) and found diffuse demyelination in the white matter localized in the corpus callosum. Hurst (1940) attempted to duplicate these results in monkeys by giving cyanide at near lethal doses daily or at longer intervals but did not obtain consistent results. If a nonlethal cyanide level was maintained in the rat for 20 to 30 min by HCN inhalation or by slow intravenous injection of KCN, the white matter was always damaged (Levine, 1967). Distended vacuoles appeared at one point on the axons that crossed the corpus callosum (Hirano, Levine, and Zimmerman, 1967). Lesions first appeared and were more severe in an area of white matter. Smith et al. (1963, cited in Smith, 1964) showed that comparatively small doses of cyanide given at long intervals produced histological changes in the central nervous system of the rat.

From experiments on mice, Isom, Liu, and Way (1975) concluded that cyanide exposure could induce a marked alteration in normal carbohydrate metabolism and that this alteration might be associated with pathological conditions related to cyanide exposure.

6.3.3.1 Tropical Neuropathies and Amblyopias — The fully developed syndrome of tropical neuropathies is characterized by optic atrophy, nerve deafness, and sensory spinal ataxia (Money, 1958). Various fragmentary forms of the fully developed syndrome may occur. Tropical amblyopia was first studied in Nigeria by Moore (1930, 1932, 1934*a*, 1937, cited in Osuntokun et al., 1968). Cyanide was suggested as a possible contributing factor by Clark (1935), Monekosso and Annan (1964), Monekosso and Wilson (1966), and Osuntokun (1968). The diet of many people in tropical areas

TABLE 6.6. ANIMAL RESPONSE TO INHALED CYANIDE AND CYANIDE-CONTAINING COMPOUNDS

Compound	Animal	Cyanide concentration (mg/liter)	Cyanide concentration (ppm)	Response	Reference
Hydrogen cyanide	Rat	0.12	110	Fatal in 1.5 hr[a]	Dudley, Sweeney, and Miller, 1942
	Guinea pig	0.35	315	Fatal[a]	Dudley, Sweeney, and Miller, 1942
	Rabbit	0.35	315	Fatal[a]	Dudley, Sweeney, and Miller, 1942
	Cat	0.35	315	Respiratory paralysis, 2 min; death, 5-10 min[a]	Dudley, Sweeney, and Miller, 1942
	Dog	0.125	115	Fatal[a]	Dudley, Sweeney, and Miller, 1942
Cyanogen	Mouse	0.5	235	Recovered, 15-min exposure	Fassett, 1963
	Mouse	4.26	2000	Fatal, 13-min exposure	Fassett, 1963
	Rat	0.851	400	No deaths, 45-min exposure	McNerney and Schrenk, 1960
	Rat	0.851	400	Fatal, 60-min exposure	McNerney and Schrenk, 1960
	Rat	8.508	4000	Fatal, 15-min exposure	McNerney and Schrenk, 1960
	Rabbit	0.84	400	Fatal, 1.8-hr exposure	Fassett, 1963
	Cat	0.42	200	Fatal, 1/2-hr exposure	Fassett, 1963
Cyanogen chloride	Mouse	1.0	400	Fatal to some, 3-min exposure	Fassett, 1963
	Rat	1.40		Fatal, 10-min exposure	Spector, 1956
	Rat	2.80		Fatal, 5-min exposure	Spector, 1956
	Rabbit	3.0	1200	Fatal, 2-min exposure	Fassett, 1963
	Cat	0.3	120	Fatal, 3.5-min exposure	Fassett, 1963
	Dog	0.12	48	Fatal, 8-hr exposure	Fassett, 1963
	Dog	0.8	320	Fatal, 7.5-min exposure	Fassett, 1963
	Dog	1.0	400	Fatal, <1-min exposure	Fassett, 1963
	Goat	2.2		Fatal, 7- to 10-min exposure	Spector, 1956
Cyanogen bromide	Mouse	1	230	Fatal	Fassett, 1963
	Cat	1	230	Fatal	Fassett, 1963
Acrylonitrile	Rat		75	Fatal to 0/20, 7-hr exposure	Brieger, Rieders, and Hodes, 1952
	Rat		100	Fatal to 4/20, 7-hr exposure	Brieger, Rieders, and Hodes, 1952
	Rat	0.28	130	Slight transitory effect[a]	Dudley, Sweeney, and Miller, 1942
	Rat	1.38	635	Fatal, 4-hr exposure[a]	Dudley and Neal, 1942
	Guinea pig	0.58	265	Slight transitory effect[a]	Dudley, Sweeney, and Miller, 1942
	Guinea pig	1.25	575	Fatal during or after exposure	Dudley, Sweeney, and Miller, 1942
	Rabbit	0.29	135	Marked transitory effect[a]	Dudley, Sweeney, and Miller, 1942
	Rabbit	0.56	260	Fatal during or after exposure[a]	Dudley, Sweeney, and Miller, 1942
	Cat	0.33	153	Markedly toxic, sometimes fatal[a]	Dudley, Sweeney, and Miller, 1942
	Cat	0.60	275	Markedly toxic[a]	Dudley, Sweeney, and Miller, 1942
	Dog		50	Fatal to 0/4, 7-hr exposure	Brieger, Rieders, and Hodes, 1952
	Dog		75	Fatal to 3/4, 7-hr exposure	Brieger, Rieders, and Hodes, 1952
	Dog		100	Fatal to 6/6, 7-hr exposure	Brieger, Rieders, and Hodes, 1952
	Dog	0.213	100	Convulsions and coma, death[a]	Dudley, Sweeney, and Miller, 1942
	Dog	0.24	110	Fatal, 4-hr exposure[a]	Dudley and Neal, 1942
	Dog	0.24	110	Fatal to 3/4[a]	Dudley, Sweeney, and Miller, 1942
	Rhesus monkey		75	Fatal to 1/3, 7-hr exposure	Brieger, Rieders, and Hodes, 1952
	Monkey	0.33	153	Definite toxic effects[a]	Dudley, Sweeney, and Miller, 1942
Acetonitrile	Rat, male		7500	LC_{50}, 8-hr exposure	Fassett, 1963
	Dog		8000	No fatality, 4-hr exposure	Fassett, 1963
Isobutyronitrile	Rat		5500	Fatal, 1-hr exposure	Fassett, 1963
Propionitrile	Rat		9500	Fatal, 1.5-hr exposure	Fassett, 1963

[a] Repeated exposure for 4 hr/day, five days per week for eight weeks.

TABLE 6.7. LD$_{50}$ OF COMPOUNDS CONTAINING CYANIDE MOIETY AFTER SKIN ABSORPTION BY RABBITS AND GUINEA PIGS

Compound	Animal	Dose	Skin response	Reference
Cyanogen	Rabbit	10,000 ppm	No effect, 8-hr exposure[a]	McNerney and Schrenk, 1960
Acetonitrile	Rabbit	1.25 ml/kg		Fassett, 1963
Isobutyronitrile	Guinea pig	<5 ml/kg	Slight irritation	Fassett, 1963
Propionitrile	Guinea pig	<5 ml/kg	Slight irritation	Fassett, 1963

[a] Inhalation exposure was prevented.

TABLE 6.8. ACUTE TOXICITY OF CYANIDE AND CYANIDE-CONTAINING COMPOUNDS TO EXPERIMENTAL ANIMALS

Compound	Animal	Dosage (mg/kg)	Route of administration[a]	Effect	Reference
Hydrogen cyanide	Rabbit, male	1.50 (1.27-1.80)	IM	LD$_{50}$	Ballantyne et al., 1971
	Rabbit, female	0.95 (0.81-1.11)	IM	LD$_{50}$	Ballantyne et al., 1971
Potassium cyanide	Mouse	6.02 ± 3.3	OR	LD$_{50}$	Streicher, 1951
	Rat, male	8.7-11.5	OR	LD	Gaines, 1969
	Rabbit, male	3.06 (2.61-3.63)	IM	LD$_{50}$	Ballantyne et al., 1971
	Rabbit, female	3.27 (2.70-4.08)	IM	LD$_{50}$	Ballantyne et al., 1971
Sodium cyanide	Rat	15 (11-21)	OR	LD$_{50}$	Smyth et al., 1969
	Dog	5.36 ± 0.28	SC	LD$_{50}$	Chen and Rose, 1952
Calcium cyanide	Rat	39 (30-51)	OR	LD$_{50}$	Smyth et al., 1969
Cyanogen chloride	Mouse	39.07	SC	LD	Hunt, 1923
	Rabbit	20.038	SC	LD	Hunt, 1923
	Pigeon	43.53	SC	LD	Hunt, 1923
Cyanogen iodide	Frog	111-143	SC	LD	Hunt, 1923
	Mouse	27-36	SC	LD	Hunt, 1923
	Rat	44	SC	LD	Hunt, 1923
	Rabbit	23.5	OR	LD	Hunt, 1923
	Rabbit	19-40	SC	LD	Hunt, 1923
	Rabbit	15	IV	LD	Hunt, 1923
	Cat	18	OR	LD	Hunt, 1923
	Cat	23	SC	LD	Hunt, 1923
	Dog	19-30	SC	LD	Hunt, 1923
Acetonitrile	Rat	1.7-8.5	OR	LD$_{50}$	Fassett, 1963
	Guinea pig	0.18	OR	LD$_{50}$	Fassett, 1963
Propionitrile	Rat	50-100	OR	LD$_{50}$	Fassett, 1963
	Rat	25-50	IP	LD$_{50}$	Fassett, 1963
	Guinea pig	25-50	OR	LD$_{50}$	Fassett, 1963
	Guinea pig	10-25	IP	LD$_{50}$	Fassett, 1963
Isobutyronitrile	Mouse	5-10	OR	LD$_{50}$	Fassett, 1963
	Rat	50-100	OR	LD$_{50}$	Fassett, 1963

[a] IM — intramuscular; OR — oral; SC — subcutaneous; IP — intraperitoneal.

includes cassava, sometimes as a major component of the diet. Cassava has a high cyanogenic glycoside (linamarin) content (Section 4.2.3), which releases HCN on enzymatic or acid hydrolysis. Osuntokun, Monekosso, and Wilson (1969) compared the prevalence of neurological disorders in two Nigerian villages that differed in amount of cassava eaten (64.3% cassava meals in one village vs 10.8% in the other) but were similar with respect to the population's mean age, weight, height, and prevalence of sickling. A degenerative neuropathy occurred with a relatively high frequency in the village with high cassava consumption. It should be pointed out that factors other than cyanide such as protein deficiency, riboflavin deficiency, abnormal vitamin B_{12} metabolism, and infections may be involved. These other factors still may be related to cyanide. For example, lack of substrate for normal cyanide detoxification can occur because of the deficiency of sulfur-containing amino acids in the diet (Osuntokun et al., 1968). Most etiological studies have focused on chronic cyanide intoxication (Monekosso and Wilson, 1966; Osuntokun, 1968; Osuntokun et al., 1968; Osuntokun, Monekosso, and Wilson, 1969; Osuntokun, Aladetoyinbo, and Adeuja, 1970). From studies on 320 patients with Nigerian ataxic neuropathy, Osuntokun (1972) reported that protein-calorie deficiency and deficiency of water-soluble vitamins were not important in the etiology of the disease but contributed to the clinical picture. These patients had demyelination of peripheral nerves and resultant decreased conduction velocity of motor nerves.

Makene and Wilson (1972) concluded, from data on thiocyanate and vitamin B_{12} levels in plasma of Tanzanian patients with ataxic tropical neuropathy, that the condition may be attributed to chronic cyanide intake from cassava. Data in Table 6.9 show that thiocyanate levels were significantly higher in eight patients with ataxic neuropathy than in nine controls at the $P < 0.1\%$ level. Vitamin B_{12} levels, both with and without cyanide added to the extraction mixture, were significantly higher at the 5% level in ataxic neuropathy patients.

Other neuropathies such as West Indian amblyopia, tobacco amblyopia, and Leber's optic atrophy are all characterized by visual field defects and may be attributed to cyanide as a possible etiological factor. MacKenzie and Phillips (1968) examined the visual field of ten West Indian amblyopia patients. There is no direct evidence that cyanide was involved, however, the above authors felt that it was a possible factor. The question was raised that Leber's optic atrophy may be an inborn error of cyanide metabolism which becomes apparent when the body is confronted with a source of cyanide such as tobacco smoke, cassava, or certain infections (Wilson, 1956). It is of interest to note that in tobacco amblyopia, vitamin B_{12}, particularly as hydroxocobalamin, is an effective treatment (Chisholm, Bronte-Stewart, and Foulds, 1967). Chisholm, Bronte-Stewart, and Foulds (1967) administered to patients 1.0 mg of cyanocobalamin or hydroxocobalamin parenterally daily for two weeks, followed by 1.0 mg twice weekly for four weeks, and then 1.0 mg at monthly intervals. Figure 6.2 shows the improvement in vision with time of treatment, especially with hydroxocobalamin.

TABLE 6.9. PLASMA THIOCYANATE AND VITAMIN B_{12} LEVELS IN NEUROPATHY PATIENTS AND IN CONTROLS WITH MISCELLANEOUS DISEASES

Compound	Patients with ataxic neuropathy	Patients with miscellaneous diseases
Thiocyanate	9.3 ± 1.9 μmoles/100 ml plasma	2.8 ± 0.82 μmoles/100 ml plasma
B_{12} + CN	700 ± 118 pg/ml plasma	407 ± 60 pg/ml plasma
B_{12} − CN	380 ± 64 pg/ml plasma	227 ± 34.2 pg/ml plasma

Source: Adapted from Makene and Wilson, 1972, Table 1 and Table 2, p. 32. Reprinted by permission of the publisher.

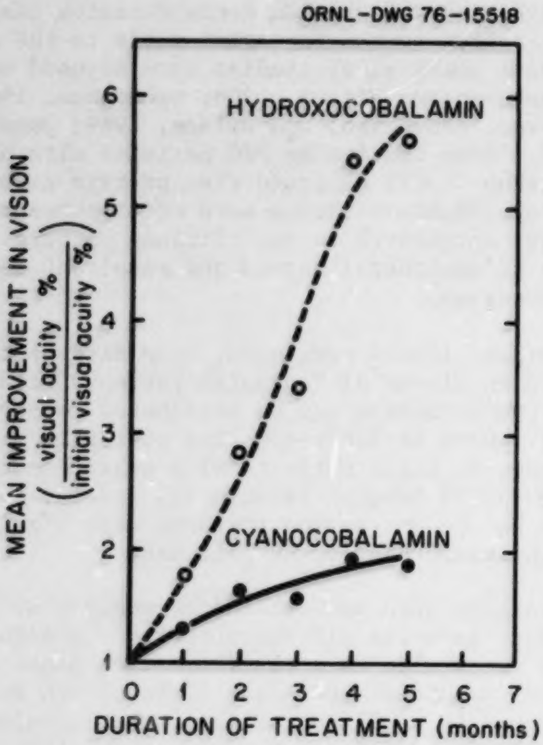

Figure 6.2. Rate of visual improvement per month in patients with tobacco amblyopia treated with parenteral hydroxocobalamin or cyanocobalamin. Source: Adapted from Chisholm, Bronte-Stewart, and Foulds, 1967, Figure 1, p. 451. Reprinted by permission of the publisher.

Earlier, Heaton, McCormick, and Freeman (1958) achieved some success in treating tobacco amblyopia with cyanocobalamin (100 mg) parenterally. The treatment was discontinued after six months if the symptoms were gone. Small amounts of hydroxocobalamin are present in commercial preparations of cyanocobalamin and may actually have been the effective substance (Smith and Duckett, 1965). Bronte-Stewart, Chisholm, and Lewis (1968) reported a case of tobacco amblyopia that did not respond to cyanocobalamin treatment but did improve when hydroxocobalamin was administered. Foulds et al. (1968) reported a similar situation in one, and possibly two, cases of Leber's hereditary optic atrophy that did not respond to cyanocobalamin but did improve with hydroxocobalamin. Because cyanocobalamin may actually accelerate development of optic atrophy, hydroxocobalamin has been specifically recommended (Foulds et al., 1970).

Cyanide has been implicated as a possible etiological agent in various human neuropathies. Lessell (1971), however, suggested caution in attributing the etiology of human nerve disorders to cyanide. Studies with rats (Lessell, 1971; Lessell and Kuwabara, 1974) indicate that nerve damage from cyanide in experimental cyanide lesions and human disorders were similar. However, the cyanide dose necessary to produce experimental nerve damage was in the lethal range. Also, in the rat the corpus callosum is much more sensitive to cyanide than is the optic nerve, whereas in human disorders ocular involvement is often the predominent or only indication of brain involvement.

6.3.3.2 Goitrogenic Action — Cassava may contribute to endemic goiter and cretinism. A high incidence of mental deficiency in many regions where endemic goiter occurs suggests a possible common etiological factor (Ermans et al., 1972). Iodine deficiency is generally considered to be involved in endemic goiter; however, in some cases it may not be the only factor (Delange, Thilly, and Ermans, 1968). A study of many communities throughout Idjwi Island in Lake Kivu (Republic of Zaire) showed a severe and uniform iodine deficiency in the whole population, but endemic goiter occurred in only one region. Cassava was eaten in especially large amounts in this region (Delange and Ermans, 1971). Thiocyanate, resulting from detoxification of cyanide released from the cassava, may be exerting an antithyroid action, as this is a well known effect of thiocyanate (Barker, 1936; Barker, Lindberg, and Wald, 1941; Ermans et al., 1972). Thiocyanate, a metabolite of the cyanogenic glycoside linamarin in cassava, can affect thyroid function, producing an endemic cretinism (Ermans et al., 1972). Although there is no direct evidence indicating that cassava consumption is responsible for endemic goiter and cretinism in this region, experimental data in rats are consistent with data from subjects in the goitrous area (Ermans et al., 1973). Ermans et al. (1973) presented the following observations:

> 1) The investigations first show that in rats, continuous intake of cassava is capable of causing changes in iodine and thiocyanate metabolism which are similar to those obtained by prolonged administration of thiocyanate.
>
> 2) Ingestion of thiocyanate or cassava entails marked depletion of iodine stores: depletion is fairly moderate in iodine-supplemented rats. This depletion is very severe in iodine-deficient rats and is associated with major changes in intrathyroidal metabolism which iodine deficiency alone is incapable of causing.
>
> 3) Chronic ingestion of thiocyanate does not necessarily cause blocking of the thyroidal iodide pump; iodine uptake by the gland seems, on the contrary, to be increased, probably due to thyrotropic stimulation triggered by iodine depletion. This does not preclude transitory inhibition during the phase of thiocyanate absorption.
>
> 4) Administration of thiocyanate or its precursors even in increasing doses does not necessarily entail a very marked rise of SCN concentration in the blood. Evidence of increased ingestion is only obtained by measurement of urinary excretion or estimation of plasma turnover of SCN.

5) The iodine depletion seems to be mainly due to an increased loss of iodine in urine related to a blockage of the tubular reabsorption of this ion by an excess of SCN.

Other naturally occurring goitrogenic agents are present in some foods (Greer, 1962). Endemic goiter has existed for a long time in Slovakia in Czechoslovakia and 70% or more of the adult women in endemic areas in this region have goiters (Podoba, Michajlovskij, and Stukovsky, 1973). An inverse correlation exists between iodine in the drinking water in the various communities and the incidence of goiter. Also, vegetables of the Brassica family, which have a goitrogenic potential, are consumed in large amounts and have a high sulfur and thiocyanate level.

6.3.3.3 Carcinogenesis, Teratogenesis, and Mutagenesis — There is a paucity of information on the carcinogenic, teratogenic, and mutagenic properties of cyanide and the available evidence warrants further examination. Cyanide has been employed as an antitumor agent in experimental animals and in humans (Brown, Wood, and Smith, 1960; Perry, 1935; Stone, Wood, and Smith, 1959). It appears to have a selective inhibitory effect on certain tumor tissues such as the Ehrlich Ascites tumor and Sarcoma-180 in mice. The regional perfusion studies of pelvic tumor with cyanide in humans apparently can be done without evidence of toxicity. In these latter studies there was no evidence of a decrease in either tumor size or number of metastasis, but the pathologic cellular changes of the tumors were encouraging.

A long-standing claim has been made for an anticancer effect by the cyanogenic glycosides (Krebs, 1970; Morrone, 1962; Navarro, 1959); however, these claims have been vigorously refuted by various laboratories (Lewis, 1977). The rationale of cancer treatment with the cyanogenic glycoside, amygdalin (also called laetrile) is a postulated selective hydrolysis of amygdalin by a β-glucosidase, to free cyanide, benzaldehyde, and sugar at the cancer site. The cyanide was then proposed to selectively attack the cancer cell which presumably is deficient in rhodanese. Normal cells, which contained rhodanese were assumed to possess adequate concentration of the sulfur donor to be able to detoxify cyanide and therefore would suffer no permanent damage.

The opponents of this rather simplistic theory have pointed out (Greenberg, 1975; Lewis, 1977) that many tumor tissues are not selectively enriched in β-glucosidase nor are they low in rhodanese. Moreover, there are numerous studies citing a lack of antitumor activity of amygdalin in model tumor systems (Hill et al., 1976; Laster and Schnabel, 1975; Levi et al., 1965; Wodinsky and Swiniarski, 1975). It is noted, however, that no large scale carefully documented study of the effectiveness of amygdalin as an antitumor agent has been carried out on human patients.

Chronic suppression of prolactin secretion in C3H/HeJ female mice by 6-methyl-8-β-ergoline-acetonitrile significantly inhibited development of mammary hyperplastic alveolar nodules and greatly reduced mammary tumor incidence (Welsch, Gribler, and Clemens, 1974).

β-Aminopropionitrile (BAPN) was teratogenic when tested in pregnant rats (Abramovich and Devoto, 1968; Barrow and Steffek, 1974; Herd and Orbison, 1966; Wilk et al., 1972) and in pregnant baboons (Steffek and Hendrickx, 1972). Wilk et al. (1972) studied the metabolism and distribution of BAPN and cyanoacetic acid (CAA), a major metabolite, in rats. The teratogenic agent was BAPN rather than CAA; the incidence of cleft palate was correlated with maternal and fetal levels of BAPN. Day 15 of gestation is the critical time for cleft palate induction and administration of BAPN on day 14 or 16 produced few or no cleft palates. Oral administration of 270 mg BAPN on day 15 led to 98% incidence of cleft palate in offspring. Six hours after BAPN administration to pregnant rats on day 15, an embryonic level of 106, 42, and 16 µg BAPN per gram of tissue corresponded to an incidence of cleft palate of 98%, 28%, and 0%, respectively. Oral CAA did not produce cleft palate although high embryonic CAA levels resulted. Rabbits, which metabolize BAPN very efficiently, had a low incidence of cleft palate in these studies, supporting BAPN as the active agent.

Barrow and Steffek (1974) also obtained teratologic and other embryotoxic effects with BAPN in rats. Day 15 of gestation was an important time for embryotoxic effects with no gross malformations occurring if BAPN was administered before day 14 although resorptions did occur. Generally, higher doses (e.g., 5000 mg/kg body weight) caused fetal resorptions. Lower doses at certain times produced various fetal abnormalities (e.g., 2500 mg/kg on days 14 to 15) such as ecotcardia, gastroschisis, and cleft palate. Complete data are shown in Table 6.10.

Pregnant baboons were given BAPN to extend the earlier studies (Steffek and Hendrickx, 1972). Only three animals were used in this preliminary study and each was given a different dose size via a different route at a different time during gestation; therefore, the teratogenic potential could not be determined. The data are presented in Table 6.11. Palate formation and closure occurs during days 40 to 50 of gestation in baboons. The effects of aminoacetonitrile (AAN) were also tested in this study. The results are difficult to interpret. The appearance of cleft palate in each twin of a dizygous set was taken as an indication of a common environmental etiology (i.e., AAN administration).

6.3.4 Treatment for Cyanide Poisoning

Because cytochrome oxidase is the most sensitive enzyme to cyanide (Section 6.3.2), any substance that can effectively compete with cytochrome oxidase for the cyanide ion could be an effective antagonist. Methemoglobin is an effective competitor (Albaum, Tepperman, and Bodansky, 1946) and the body can tolerate blood concentrations of 30% with no symptoms and up to 70% before lethal levels are reached (Bodansky, 1951). The accepted treatment for acute cyanide poisoning (Chen, Rose, and Clowes, 1933, 1934; Hug, cited in Chen and Rose, 1952) is an intravenous injection of sodium nitrite (0.3 g in 10 ml water) to convert a portion of the hemoglobin to methemoglobin, followed immediately by an intravenous injection of thiosulfate (12.5 g in 50 ml water) to supply a sulfur donor, the substrate, for rhodanese. Usually amyl nitrite is inhaled by the patient

TABLE 6.10. EMBRYOLETHAL AND TERATOLOGIC EFFECTS OF BAPN IN PREGNANT RATS

Days treated	Dose (mg/kg)	Number of litters	Resorptions		Number of living fetuses	Abnormalities									
						Ectocardia	Gastroschisis	Both	Total		Cleft palate		Osteolathyritic effects		
			No.	%					No.	%	No.	%	No.	%	Degree
0 (controls)		111	79	7	1124	0	0	0	0	0	0	0	0	0	
1-7	1000	10	3	3	83										
8-13	1000	15	74	54	64										
9	3500, 4250, 5000	9	12	16	63										
10	3000, 3500, 4250	15	12	9	116										
11	3500, 5000	12	34	43	45										
11-12	4250	4	27	100											
11-16	1000	19	78	47	87						24	27	87	100	Mild to moderate
11-17	1000	4	14	34	27						1	4	27	100	Mild
11-17	4000	5	50	100											
11-18	1000	6	40	69	18						14	78	18	100	Mild
11-18	2000	3	31	100											
11-20	250	3	9	29	22								22	100	Very mild
11-20	500	5	31	55	25								25	100	Mild
12	3000, 3500, 4250	15	29	25	89										
12	5000	5	43	81	10								10	100	Very mild
13	2500, 3000, 3500, 4250	24	42	23	139										
13	5000	6	35	61	22								6	27	Very mild
13-14	3000	8	42	47	48	2	1		3	6	1	2	11	23	Mild
13-14	3500, 4000	9	76	100											
13-15	2500	12	63	47	70	5	3	1	9	11	7	10	3	4	Very mild
13-16	1000	7	8	14	51						19	37	51	100	Mild
13-17	1000	5	4	8	43						22	51	43	100	Moderate
13-17	4000	5	21	100											
13-18	1000	8	50	66	26						23	88	26	100	Moderate to severe

(continued)

TABLE 6.10 (continued)

Days treated	Dose (mg/kg)	Number of litters	Resorptions		Number of living fetuses	Abnormalities									
						Ecto-cardia	Gastro-schisis	Both	Total		Cleft palate		Osteolathyritic effects		
			No.	%					No.	%	No.	%	No.	%	Degree
13-20	1000	8	78	96	3						3	100	3	100	Severe
14	2500	6	8	12	56								15	27	Very mild
14	3000	5	3	6	44						3	7	8	18	Very mild
14	3500	6	60	85	11								11	100	Very mild
14	4000	5	14	34	27				1	3	1	3	9	33	Very mild
14	4500	4	20	53	18								8	44	Mild
14	5000	9	54	47	60				1	2	7	12	16	27	Mild
14	5500, 6000	7	73	95	4										
14-15	2500	30	188	55	154	11	3	11	25	16	4	4			
14-15	3000	11	87	81	21	4			4	19			6	29	Mild
14-15	3500	12	91	93	7					14	3	43	9	100	Mild
14-15	4000	9	89	100											
15	2500	13	4	4	96								10	10	Mild
15	3500	6	16	31	36	1			1	3	5	14	26	72	Mild
15	4000	15	95	68	44	1		1	2	5	12	27			
15	4500	8	66	72	26	1			1	4	4	16			
15	5000	4	36	100											
15-16	3000	3	27	96	1						1	100	1	100	Mild
15-17	2000	3	13	43	17								17	100	Mild
16	1000	3	3	9	27										
16	2500	24	112	42	152						144	95	130	86	50 severe, 80 mild
16	3000	8	40	43	54						54	100	54	100	44 severe, 10 mild
16	3500	8	52	65	28						28	100	28	100	Severe
16	4000	6	36	66	18						17	94	18	100	Severe
16	4500	3	20	50	20						20	100	20	100	Severe
16-17	1000	10	8	8	91						16	18	80	100	42 mild, 38 severe
16-17	2000	4	22	55	18						18	100	18	100	Severe
17	1000	4	1	3	30								30	100	Mild
17	2500	7	37	50	37								35	95	25 severe, 10 mild
17	3500	3	17	68	8								8	100	Severe
18	2500	4	28	72	11								11	100	Mild
19	2500	11	45	47	51								51	100	16 severe, 35 mild
19	2500	5	0	0	39										
21	2500	2	0	0	14										
	Total	480[a]													

[a] Does not include controls.

Source: Adapted from Barrow and Steffek, 1974, Table 1, pp. 168-169. Reprinted by permission of the publisher.

as an emergency means of inducing methemoglobinemia because it can be given immediately while sodium nitrite and thiosulfate injections are being prepared. Data from Chen and Rose (1952) showed the comparative effects of these compounds alone and in combination on cyanide poisoning in dogs (Table 6.12).

TABLE 6.11. EFFECT OF BAPN AND AAN ON FETAL DEVELOPMENT IN BABOONS

Drug	Days of treatment during gestation	Dose (mg kg^{-1} day^{-1})	Route of administration[a]	Results
BAPN	38-50	200	IM	Fetal resorption
	37-48	300	OR	Fetal resorption
	43-48	500	IV	Fetal maceration, spina bifida
AAN	38-50	20	IM	Abortion
	38-50	20	IM	Normal
	38-50	40	IM	Normal
	38-41	70	IM	Abortion
	38-48	60	OR	Fetal resorption
	40-48	60	OR	Normal
	43-48	75	OR	Abnormal flexure of digits on right foot
	43-48	75	OR	Twins, both with cleft palate and abnormal curvature of arms and legs
	45-48	130	OR	Normal
	46-48	130	OR	Normal

[a] IM — intramuscular; OR — oral; IV — intravenous.

Source: Adapted from Steffek and Hendrickx, 1972, Table 1, p. 172. Reprinted by permission of the publisher.

TABLE 6.12. COMPARISON OF EFFECT OF ANTIDOTES ON CYANIDE POISONING IN DOGS

Antidote	Number of dogs	LD_{50} of sodium cyanide ± standard error (mg/kg)	Ratio of LD_{50}'s of sodium cyanide in treated and untreated dogs
None	16	5.36 ± 0.28	1
Sodium thiosulfate	11	18.4 ± 0.9	3
Amyl nitrite	13	24.5 ± 1.2	5
Sodium nitrite	13	27.1 ± 3.1	5
Amyl nitrite and sodium thiosulfate	17	60.9 ± 3.0	11
Sodium nitrite and sodium thiosulfate	21	96.7 ± 23.6	18

Source: Adapted from Chen and Rose, J. Am. Med. Assoc., May, Vol. 149, Table 1, p. 114, copyright 1952, American Medical Association.

It would seem reasonable to employ oxygen with the classic nitrite-thiosulfate therapy, as oxygen appears to enhance the antidotal effect of this combination (Sheehy and Way, 1968; Way, Gibbon, and Sheehy, 1966*a*, 1966*b*; Way et al., 1972). Also various cobalt containing compounds are employed as a cyanide antagonist (Burrows and Way, 1977; Evans, 1964; Friedberg, Grutzmacher, and Lendle, 1965; Isom and Way, 1973; Mushett et al., 1952; Paulet, 1958; Rose et al., 1965).

SECTION 6

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SECTION 7

ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

7.1 SUMMARY

Production figures for cyanide are over 700 million pounds per year and are steadily increasing. Figures indicating increased yearly production are available for dicyandiamide, acrylonitrile, and other organic nitriles. The primary users of cyanide compounds include the electroplating, steel, plastics, and various chemical industries.

Cyanide wastes are produced by various industrial processes. Some attempts have been made to control waste cyanide but an appreciable amount is still being discharged into the environment. Sources of cyanide that enter the environment include the steel and electroplating industries, mining operations, catalytic converters on automobiles, home fires, and hospital laboratories.

Data concerning cyanide movement in soils are fragmentary. Cyanide is believed to not be strongly adsorbed or retained by soils. Since most cyanide salts are soluble, they move through soils and are converted to other compounds or are fixed by trace metals.

Cyanides are believed to be relatively uncommon in most U.S. water supplies. When they do occur, they are usually less than the U.S. Public Health Service limit. Cyanides in the atmosphere probably are increasing as a result of pollution. However, data concerning the distribution and transformation of cyanides in air are lacking.

Of the many processes which have been proposed for cyanide removal from industrial wastewater, only a few appear to be economically feasible and are commonly used (e.g., alkaline chlorination, electrolytic decomposition, and ozonation). Improper management of cyanide wastes has led to various incidents of environmental damage.

7.2 PRODUCTION AND USAGE

Information concerning the level of cyanide production has been regarded as confidential since 1939 (Fairhall, 1969). Only fragmentary production figures are available. The estimated U.S. production capacity for hydrogen cyanide varies from different sources. It was 202 million kilograms (445 million pounds) as of early 1964 (Montgomery, 1965) and 700 million pounds in 1976. Of this amount, 52% was used in acrylonitrile production, 18% in methyl methacrylate production, 14% in adiponitrile production, and 7% in sodium cyanide production; the remaining 9% was used for various purposes.

The world production capacity (excluding the USSR and its satellites) for sodium cyanide is greater than 90,800 metric tons (100,000 tons) per year (Mooney and Quin, 1965). Actual usage in 1963 was about

64,000 to 73,000 metric tons (70,000 to 80,000 tons). World production of potassium cyanide was about 4500 metric tons (5000 tons). Free world production of dicyandiamide was 90,800 metric tons (100,000 tons) in 1962 (McAdam and Schaefer, 1965). Melamine production consumed 80% to 90% of this output. Recent U.S. production of acrylonitriles has been about 5 x 10^5 metric tons and has grown at about 12% per year (National Academy of Sciences, 1975). Exports in 1972 were 2470 tons, about 0.5% of the total production. Imports amounted to 1.0 metric ton (1.1 tons).

The primary uses of some major cyanide compounds are given in Table 7.1. A large percentage of cyanide usage is by the electroplating, steel, and chemical industries.

7.3 SOURCES

Cyanide wastes are produced by various industries, and only a relatively small fraction of cyanide is believed to escape into the environment. The largest amount of cyanide wastes is generated by the electroplating industry (Table 7.2). Because cyanides are such good complexing agents, they are used in plating baths. The concentration of cyanides in liquid wastes of the National Association of Metal Finishers member plants ranges from 9 to 115 ppm, with an average of 72 ppm (Reed et al., 1971). Waste streams of the electroplating industry may contain 0.5% to 20% cyanide before treatment. Approximately 9,660,000 kg (21,300,000 lb) of cyanide wastes is discharged each year by 2600 U.S. electroplating plants (Table 7.3).

Paint manufacturing generates cyanide wastes. About 20,000 kg (45,000 lb) of cyanide is lost each year through 16.8 million kilograms (37 million pounds) of solvent-based waste paint sludges (Ottinger et al., 1973*a*). Additionally, residues in used paint containers provide an estimated 141,000 kg (310,000 lb) of cyanide each year to the environment.

The steel industry also provides cyanide input. Weak ammonia liquor from the Bethlehem Steel Corporation results from coking 4350 metric tons (4800 tons) of coal per day and contains 20 to 80 ppm cyanide as CN^- and 700 to 1300 ppm as thiocyanate (Cousins and Mindler, 1972). Typical coke oven liquor contains 6 ppm cyanide, with a range of 0 to 8 ppm (Pearce and Punt, 1975). A liter of coke oven gas normally contains about 2.3 mg of HCN (0.5 to 1 grain per standard cubic foot) (Mitachi, 1973).

Cyanide enters the environment as a result of mining operations. Raw wastewaters from copper mine and concentrator operations contain 0.06 ppm cyanide (Hallowell et al., 1973). The Tjikotok mineral processing plant in West Java discharges 1.25 x 10^8 kg of waste (containing about 800 mg sodium cyanide per kilogram) per year to the river Tjimadur (Bowen, 1971).

Laboratory wastes provide a minor cyanide emission. Hospital labs in Buffalo, New York, have discharged 5600 g of cyanide per year to the environment (Pragay, 1974). It is estimated that a laboratory in a 1000-bed hospital in the Buffalo area would discharge 930 g of cyanide per

TABLE 7.1. PRIMARY USES OF SOME MAJOR CYANIDE COMPOUNDS

Compound	Uses	Reference
Acrylonitrile, C_2HN	Production of acrylic and modacrylic fibers, nitrite elastomers, plastics	National Academy of Sciences, 1975
Cadmium cyanide, $Cd(CN)_2$	Electroplating	Ottinger et al., 1973*b*
Calcium cyanide, $Ca(CN)_2$	Ore cyanidation, froth flotation, fumigation, HCN production, ferrocyanide production, rodenticide, case hardening of steel, cement stabilizer	National Academy of Sciences, 1975
Calcium cyanamide, $CaCN_2$	Fertilizer, defoliant, weed killer, production of melamine, steel production	McAdam and Schaefer, 1965
Cuprous cyanide, CuCN	Electroplating, medicine, insecticide, oxygen removal from molten metals, underwater paint, organic nitrile separation	Ottinger et al., 1973*b*
Cyanogen, C_2N_2	Fumigant	Hardy and Boylen, 1971
Cyanogen bromide, CNBr	Organic syntheses, fumigant, pesticide, gold extraction, cellulose technology	Mooney and Quin, 1965
Dicyandiamide, $C_2N_2(NH_2)_2$	Melamine manufacture, vinyl resin stabilizer, curing agents for epoxy resins, textile-drying assistant, starch fluidifying agent, guanidine salt production	McAdam and Schaefer, 1965
Hydrogen cyanide, HCN	Rodenticide, insecticide, electroplating, ethyl lactate, acrylonitrile synthesis, ferrocyanide manufacture, lactic acid, chelating agents, optical laundry bleaches, pharmaceuticals	Hardy and Boylen, 1971; Montgomery, 1965
Lead cyanide, $Pb(CN)_2$	Insecticide, electroplating	Ottinger et al., 1973*b*
Melamine, $C_3N_3(NH_2)_3$	Melamine-formaldehyde resins, textile fire retardants, bactericide, tarnish inhibitor	McAdam and Schaefer, 1965
Nickel cyanide, $Ni(CN)_2 \cdot 4H_2O$	Electroplating	Ottinger et al., 1973*b*
Potassium cyanate, KOCN	Weed killer, chemical intermediate	Zuzik, 1974
Potassium cyanide, KCN	Electroplating, steel hardening, extraction of metals from ores, nitrile manufacture, fumigation, photography, silver polish	Hardy and Boylen, 1971; Hamilton and Hardy, 1974
Potassium ferricyanide, $K_3Fe(CN)_6$	Photography, blueprints, metal tempering, electroplating, pigments	Hardy and Boylen, 1971

(continued)

TABLE 7.1 (continued)

Compound	Uses	Reference
Potassium ferrocyanide, $K_4Fe(CN)_6 \cdot 3H_2O$	Tempering of steel, process engraving, pigment manufacture, dyes	Hardy and Boylen, 1971
Silver cyanide, AgCN	Electroplating	Ottinger et al., 1973*b*
Sodium cyanate, NaOCN	Organic syntheses, heat treatment of steel, pharmaceuticals	Hardy and Boylen, 1971
Sodium cyanide, NaCN	Metal treatment, electroplating, synthesis of organic intermediates, ore extraction, organic chemical synthesis, photography, silver polish	Hamilton and Hardy, 1974; Ottinger et al., 1973*b*
Zinc cyanide, $Zn(CN)_2$	Medicine, electroplating	Ottinger et al., 1973*b*

TABLE 7.2. TYPICAL ELECTROPLATING WASTES CONTAINING CYANIDES

Waste description
Liquid waste containing 15% sodium cyanide, a 10% mixture of sodium ferrocyanide and sodium ferricyanide, and traces of nickel and zinc
Stripping solution containing 13% sodium cyanide, sodium hydroxide, and 600 ppm copper
0.8% cyanide, 3300 ppm zinc, 165 ppm nickel, and trace of silver in a 1% sodium hydroxide solution
Slurry containing 20% sodium ferrocyanide, 2% zinc and insoluble material, and 50% water
Slurry containing 2.5% zinc ferrocyanide, 2% calcium fluoride, 3% chromic hydroxide, and 80% water
1% potassium ferrocyanide and <50 ppm lead, nickel, chromium, and copper combined in an aqueous 10% sodium hydroxide solution
3% to 5% sodium cyanide and 1% to 3% nickel, cadmium, copper, and zinc in an aqueous 10% sodium hydroxide solution

Source: Modified from Ottinger et al., 1973*c*, Table 3, p. 23.

year. A similar hospital in the Rochester area would discharge 3500 g. The cyanide is generated mostly during hemoglobin and uric acid determinations.

Less than 908 kg (2000 lb) of cyanide compounds is awaiting disposal in Department of Defense storage facilities. These compounds include sodium, calcium, copper, potassium, and silver cyanides, and potassium ferrocyanide, and potassium ferricyanide. All except calcium cyanide were acquired for plating purposes.

7.4 DISTRIBUTION AND TRANSFORMATION IN THE ENVIRONMENT

7.4.1 Distribution and Transformation in Soils

Data concerning the distribution and movement of cyanides in soil are limited and fragmentary. Because the toxicity of cyanide is well known, treatment of wastes is a standard practice. High concentrations in the soil are unusual and are nearly always the result of improper waste disposal (Section 7.5). Although many herbicides contain a nitrile group and are directly applied to soils, the cyanide group is not believed to be released from these compounds during breakdown and, thus, is not responsible for herbicidal activity. Since herbicidal activity is appar-

TABLE 7.3. INORGANIC CYANIDE WASTES

Source and material	Bureau of the Census regions									Total
	I	II	III	IV	V	VI	VII	VIII	IX	
					Annual waste production (lb/year)					
Cyanides from electroplating	2.78 x 10^6	6.07 x 10^6	6.86 x 10^6	0.96 x 10^6	1.04 x 10^6	0.49 x 10^6	0.77 x 10^6	0.15 x 10^6	2.20 x 10^6	21.32 x 10^6
Paint sludge										
cyanides	1,100	9,900	13,800	2,900	3,850	2,150	3,350	550	7,300	44,900
sludge	0.92 x 10^6	8.12 x 10^6	11.32 x 10^6	2.40 x 10^6	3.16 x 10^6	1.76 x 10^6	2.74 x 10^6	0.44 x 10^6	5.97 x 10^6	36.83 x 10^6
Paint residue										
cyanides	0.18 x 10^5	0.57 x 10^5	0.62 x 10^5	0.23 x 10^5	0.47 x 10^5	0.20 x 10^5	0.30 x 10^5	0.13 x 10^5	0.41 x 10^5	3.11 x 10^5
old paint	13 x 10^6	41 x 10^6	44 x 10^6	16 x 10^6	34 x 10^6	14 x 10^6	21 x 10^6	9 x 10^6	29 x 10^6	221 x 10^6
					Stored wastes (lb)					
Sodium cyanide		1,400						16		1,416
Calcium cyanide							180		25	205
Copper cyanide		100						32		132
Potassium cyanide					2					2
Silver cyanide								16	10	26
Potassium ferricyanide					4					4
Potassium ferrocyanide						12				12

Source: Ottinger et al., 1973*a*, Table 1, p. 135.

ently related to the integrity of the whole molecule, these herbicides are not herein discussed.

Cyanide ions are not strongly adsorbed or retained by soils (Murrmann and Koutz, 1972). The cyanide salts of most cations are soluble (except AgCN) but move only a short distance through soil before being biologically converted under aerobic conditions to nitrates (microbial degradation to NH_3, then conversion to NO_3^-; Section 3.2.2.2) or fixed by trace metals through complex formation. Under anaerobic conditions, cyanides denitrify to gaseous nitrogen compounds which enter the atmosphere. The cyanide ion is not involved in oxidation-reduction reactions (Murrmann and Koutz, 1972).

The carbon and nitrogen of cyanide are converted to carbonate and ammonia, respectively, in nonsterile soils (Strobel, 1967). Doubly labeled cyanide ($^{14}C^{15}N$) has shown that retention of the cyanide nitrogen by soil is greater than retention of the cyanide carbon. Compounds such as cyanamid, thiourea, dicyandiamide, guanidine nitrate, guanylurea, and uramon are rapidly converted to ammonium and ultimately nitrate when they are applied to soils as fertilizers at rates up to 100 ppm (Fuller, Caster, and McGeorge, 1950).

7.4.2 Distribution and Transformation in Water

Cyanides occur in water as (1) free hydrocyanic acid (HCN), (2) simple cyanides (alkali and alkaline earth cyanides), (3) easily decomposable complex cyanides such as $Zn(CN)_2$, and (4) sparingly decomposable complex cyanides such as $[Fe^{3+}(CN)_6]^{3-}$, $[Fe^{2+}(CN)_6]^{4-}$, and $Co(CN)_4$. Complex nickel and copper cyanides assume an intermediate position between the easily decomposable and sparingly decomposable compounds (Leithe, 1973). The recommended limit for cyanide in U.S. waters is 10 ppb; concentrations of 200 ppb and above constitute grounds for rejection of the water supply (U.S. Department of Health, Education, and Welfare, 1962).

Concentrations of cyanide exceeding the mandatory limit are usually a result of improper waste management (Section 7.5). A survey of 969 U.S. public water supply systems revealed no cyanide concentrations above the mandatory limit (McCabe et al., 1970). In 2595 water samples, the highest cyanide concentration found was 8 ppb and the average concentration was 0.09 ppb. All but one sample of United Kingdom waters contained less than 50 ppb cyanide (Reed and Tolley, 1971). The exception contained 0.1 ppm.

7.4.3 Distribution and Transformation in Air

Volatile cyanides (e.g., hydrogen cyanide) are not normal atmospheric contaminants. These compounds occur in air only occasionally via emission from plating plants, fumigation, or other special operations (Stern, 1968). As a result, no data were found concerning the distribution and transformation of cyanides in the atmosphere.

7.5 WASTE MANAGEMENT

Due to the great toxicity of most cyanide compounds, the elimination of cyanide from wastewaters is standard practice. The three main categories of removal techniques are (1) complete destruction of the cyanide ion, (2) conversion of the cyanide ion to the cyanate ion, and (3) conversion of the cyanide ion to some other less toxic form such as ferrocyanide (Reed et al., 1971).

The most frequently used method of cyanide destruction is alkaline chlorination. Wastewaters are treated with chlorine gas in an alkaline solution to oxidize cyanide usually to carbon dioxide (carbonate ion) and nitrogen (Section 2.2.1.4). If desired, the reaction may be controlled to oxidize cyanide only to cyanate (Lawes, 1972; Watson, 1973).

Hypochlorites may also be used to destroy cyanides (Section 2.2.1.4). This method involves essentially the same reactions as alkaline chlorination. The active ingredient may be supplied as sodium hypochlorite, calcium hypochlorite, or bleaching powder (Green and Smith, 1972; Watson, 1973). Other possible methods of destruction include acidification, reaction with aldehydes, electrolytic decomposition, ionizing radiation, and heating (Lawes, 1972; Ottinger et al., 1973*b*; Watson, 1973).

The acute toxicity of cyanate ion is about a thousand times less toxic than the cyanide ion, and hence, may be discharged to the environment in low concentrations in some areas. The conversion uses chlorine gas in a reaction similar to alkaline chlorination. Hypochlorites are also used. Other oxidants proposed for the conversion of cyanide to cyanate include ozone, kastone (peroxygen), and permanganate (Green and Smith, 1972; Lawes, 1972; Ottinger et al., 1973*b*; Watson, 1973).

Another method for converting cyanide to other less toxic forms is the use of iron salts. The salt, usually ferrous sulfate, complexes with free cyanide in aqueous solution and causes it to precipitate. This method is commonly used in Europe but not in the United States (Ottinger et al., 1973*b*; Watson, 1973).

Other methods described in the literature for cyanide waste treatment include complexation by polysulfides or nickel salts, ion exchange, evaporation, incineration, dilution, lagooning, and biological destruction (Avery and Fries, 1975; Cousins and Mindler, 1972; Green and Smith, 1972; Lutin, 1970; Murphy and Nesbitt, 1964; Muzzarelli and Spalla, 1972; Ottinger et al., 1973*b*; Reed et al., 1971).

Improper management of cyanide wastes can result in damage to plant and animal life. Contamination may occur as a result of improper storage, handling, or disposal of cyanides. A landfill near Denver, Colorado, has leaked cyanides to the surrounding area. Tests of surface drainage have indicated the presence of cyanide in ponded water downstream from the site. According to the site operator, significant amounts of cyanide were discharged into pits at the disposal site (U.S. Environmental Protection Agency, 1974).

Cyanide, unknowingly released from a sewage plant in Oak Ridge, Tennessee, was responsible for the death of 4800 fish in Melton Hill Lake near the sewage outfall. The source of the cyanide was not known, but it was believed to be from a metal-plating industry (Anonymous, 1975).

Disposal of cyanide wastes in a gravel pit near Cologne, Germany, resulted in groundwater contamination by hexacyanoferrate of potassium. Fortunately, tests revealed that this salt was relatively innocuous, so the water supply was not shut down (Effenberger, 1964).

About 1500 55- and 30-gal drums containing cyanides disposed of near Byron, Illinois, resulted in long-range environmental damage and livestock death. Surface water runoff from the area contained up to 365 ppm cyanide. Measures have been taken to prevent further contamination (U.S. Environmental Protection Agency, 1975).

7.6 BIOMAGNIFICATION AND CYCLING

There is no report of cyanide biomagnification within the food chain. Cyanide is not likely to accumulate in food webs because low doses are rapidly detoxified by most species and large doses result in death (Section 5). Data concerning the cycling and transformation of cyanide in the environment were not found.

7.7 CYANIDE IN FOODS

United States tolerances for HCN residues on foods are given in Table 7.4. No data for cyanide residues on processed foods were noted. A discussion of the cyanide compounds which occur naturally in edible plants can be found in Section 4.2.

TABLE 7.4. TOLERANCES FOR CYANIDE RESIDUES ON FOODS

Food	Tolerance (ppm)	Reference
Beans (dried)	25	U.S. Environmental Protection Agency, 1969
Cocoa beans	25	U.S. Environmental Protection Agency, 1969
Nuts	25	U.S. Environmental Protection Agency, 1969
Citrus fruits	50	Rules and Regulations, 1971*a*
Grains	75	Rules and Regulations, 1971*b*
Spices	250	U.S. Environmental Protection Agency, 1969

SECTION 7

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SECTION 8

ENVIRONMENTAL ASSESSMENT OF CYANIDE

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8.1 PRODUCTION, USES, TRANSPORTATION, AND POTENTIAL ENVIRONMENTAL CONTAMINATION

8.1.1 Production

The estimate for the U.S. production of hydrogen cyanide was approximately 700 million pounds in 1976, and industrial production has increased annually in the past decade. Most of the inorganic cyanides, such as the sodium and potassium salts, are prepared from hydrogen cyanide.

8.1.2 Preparations and Transportation

Hydrogen cyanide is sold as a gas or as a technical grade preparation, containing 2%, 4%, 10%, or 96% to 99.5% hydrogen cyanide. A stabilizer, such as phosphoric acid, is usually present to minimize its spontaneous polymerization and associated explosive consequences. This material is normally transported in tanks, 75-lb cylinders, steel drums, or 5-lb bottles. Hydrogen cyanide is transported predominantly by rail and to a lesser extent by trucks and barges. The Interstate Commerce Commission considers the liquid and the solid forms of cyanide to be class B poisons.

8.1.3 Uses

The predominant users of cyanide are the steel, electroplating, mining, and chemical industries. It is used as an intermediate for the manufacturing of various plastics, synthetic fibers, inorganic salts, and nitrites. Cyanide is also used in photographic development, in the fumigation of various vehicles and buildings (for rodent control), and in agriculture. The predominant plastic synthesized from hydrogen cyanide is acrylonitrile.

8.1.4 Potential Environmental Contamination

8.1.4.1 Natural Causes — The liberation of hydrogen cyanide into our environment is not totally due to human activity. There are various natural sources which could contribute hydrogen cyanide to our environment. The extent of these contributions is difficult to assess. There is a possibility that long before humans inhabited the earth, hydrogen cyanide in our atmosphere may have been higher than at the present time. Cyanide has been observed in the atmospheres of the sun and other relatively cool stars, particularly the carbon stars. Hydrogen cyanide is the second polyatomic organic molecule detected historically in interstellar space by the use of microwave absorption spectroscopy. It is considered to be one of the more important precursors for the abiotic formation of amino acids, purines, and pyrimidines. Chemical emission studies have suggested that the original heteropolypeptides on earth may have been synthesized spontaneously from hydrogen cyanide and water without the intervening formation of amino acids. The formation of cyanide in the atmosphere is not unreasonable when one realizes that some of the present methods for the production of hydrogen cyanide are the interaction of ammonia with methane and the interaction of nitric oxide with various hydrocarbons. Hydrogen cyanide can be produced and excreted from various plants, fungi, and bacteria. Also, some plants release cyanide when they are crushed or macerated.

8.1.4.2 Human Related

8.1.4.2.1 Water — Regarding the discharge of cyanide waste into our waterways, the Federal Water Pollution Act (PL 92-500) includes cyanide in the effluent quality standards. Cyanide wastes are produced by a variety of industries; however, only a small fraction of the cyanide will escape into the environment if adequate waste treatment procedures are instituted. The electroplating industry uses a considerable amount of cyanide, and it is estimated that over 20 million pounds of cyanide waste ultimately is discharged each year. Prior to treatment of this waste, the cyanide content is approximately 0.5% to 20%. The steel industry also is a potentially high contamination source. It is of significance to note that the Environmental Protection Agency (EPA) filed a refuse act suit against the ARMCO Steel Company for discharging pollutants containing cyanide into Houston, Texas, shipping channels. This discharge was in the form of a continuous-process generated effluent stream. The case of the United States versus ARMCO Steel Company (C.A. 70-H-1335) was prosecuted successfully by the EPA. Other sources of cyanide entering the environment would be from various mining operations, paint manufacturing processes, electroplating, and to a lesser extent, photographic laboratories.

8.1.4.2.2 Air — There are various potential sources for the release of hydrogen cyanide in air. One source would be the atmospheric emissions of hydrogen cyanide from the petrochemical industries. Another recent source would be from cars equipped with malfunctioning catalytic converters, as Bell Laboratory has reported that a mixture of nitric oxide, carbon monoxide, and hydrogen can produce varying amounts of hydrogen cyanide. However, due to the sulfur content of gasoline and the water content of automobile exhaust, the amount of hydrogen cyanide formed is believed to be relatively low. In a closed environment, the maximum emission level of hydrogen cyanide from raw exhaust is approximately 10 ppm. In an open environment under extremely adverse conditions, the vehicle equipped with a three-way catalyst should not raise the hydrogen cyanide level over 1.1 ppm.

Another source of hydrogen cyanide in air which does not receive much attention is from home fires. With the increased use of plastics in homes, there is a potential for the combustion of various plastic materials which may liberate hydrogen cyanide. It is generally recognized that certain plastics, such as polyurethane, will liberate hydrogen cyanide upon pyrolysis. The amount of hydrogen cyanide formed is dependent upon the conditions under which pyrolysis occurs. Lastly, probably one of the major sources of atmospheric hydrogen cyanide affecting man is tobacco smoke. It should be emphasized that the use of low tar, low nicotine, or filter cigarettes does not necessarily reduce the hydrogen cyanide concentration in cigarette smoke.

8.1.4.2.3 Foods — Another potential source of hydrogen cyanide exposure affecting man and animals results from the ingestion of substances which either contain or liberate cyanide. The fumigation of various foods may result in a cyanide residue that can persist for an extensive time period. The United States lists tolerances for hydrogen cyanide residue on various food products. These tolerances vary from 25 ppm in beans to 250 ppm in spices. There are various edible plant products containing naturally occurring substances which can release cyanide. Many of these plants contain cyanogenic glycosides and therefore can potentially liberate cyanide. This is of particular concern because some of these plants comprise a major dietary constituent in various countries. Toxicity of these cyanogenic plants is also a problem for various range animals and wildlife. Poisoning of these herbivores is more prevalent under drought conditions when these animals become less selective in their source of forage. Also, dry growing conditions have been reported to enhance the development of higher concentrations of cyanogenic glycosides in certain plants. The effect of ingestion of low levels of cyanide on a long-term basis is an area of study warranting further investigation.

8.2 ENVIRONMENTAL PERSISTENCE

8.2.1 Biomagnification and Cycling

Cyanide is a nucleophilic agent which is readily metabolized. When one considers the high toxicity of cyanide in combination with its chemical reactivity and rapid biotransformation, it is not surprising that there have been no reports of biomagnification or cycling of cyanide.

8.2.2 Persistence in Foods

When various food products are fumigated with cyanide, a cyanide residue may persist. Feeding these fumigated foods to laboratory animals results in an increased excretion of urinary thiocyanate (a major metabolite of cyanide).

8.2.3 Persistence in Soils

There are very few studies on the persistence of cyanide in soil. Usually the movement of cyanide in soil is quite limited because it is either complexed by trace metals or metabolized by various microorganisms. If a high concentration of cyanide is found in the soil, it usually can be attributed to improper industrial waste management procedures.

8.2.4 Persistence in Water

The U.S. Public Health Service recommends a limit for cyanide in U.S. water of 10 ppb (and EPA indicates a limit of 5 μg/liter). Unless the cyanide is complexed with metals, most of it will exist as free hydrogen cyanide, and an appreciable amount may be either volatilized into the atmosphere or converted into other compounds by various organisms. Because of these various factors, concentration of cyanide exceeding the mandatory limits in water usually can be attributed to improper waste disposal.

A recent survey of U.S. public water supplies revealing no concentration of cyanide above the mandatory limits is not reassuring in view of the factors previously discussed, as high pulses of cyanide from various industries into our waterways can occur without detection unless a continuous monitoring system is used.

8.2.5 Persistence in Air — Under normal circumstances, hydrogen cyanide is present in the atmosphere in sufficiently low concentrations to not be detected by standard procedures. The emission of hydrogen cyanide into the atmosphere usually can be attributed to electroplating or fumigation operations or to the pyrolysis of various plastics. There was some concern that in addition to the gas-phase hazard, the airborne water droplets generated by the combustion processes could trap enough hydrogen cyanide to exert deleterious effects. An example would be the combustion of polyvinyl chloride where the hydrogen chloride gas released can be trapped in water droplets in sufficient concentration to be of concern. However, in the atmosphere around fire, the hazards of respirable water droplets exposed to hydrogen cyanide is relatively minimal when compared to the toxicity of the gas-phase hydrogen cyanide.

8.2.6 Waste Management

The disposal of cyanide in wastewater is still a significant problem for various industries with respect to the effluent quality standards in the Federal Water Pollution Act (PL 92-500). The most common method used in management of cyanide wastewater in this country is alkaline chlorination where the cyanide is oxidized to carbon dioxide and nitrogen. In some European countries, the cyanide is complexed to iron salts as an alternative method. The mere fact that a variety of methods are being described in the current literature for cyanide waste treatment suggests that cyanide disposal still is a significant problem to some industries. Other methods employed in cyanide waste treatment include complexation with various metals, biologic transformation by cyanide-resistant microorganisms, ionizing radiation, ion exchange processes, ozonation, charcoal adsorption, and reverse osmosis.

8.3 EFFECTS OF AQUATIC AND TERRESTRIAL ORGANISMS

8.3.1 Aquatic Organisms

The National Academy of Sciences and the National Academy of Engineering in 1972 indicated that 0.005 ppm of cyanide should represent a maximal safe concentration in water containing aquatic life. Also, these academies indicated that 0.01 ppm or higher is hazardous to the marine environment. This is of interest because the recommended limit for cyanide in U.S. waters is 0.01 ppm and concentrations exceeding 0.2 ppm constitute grounds for rejection of the water supply (U.S. Department of Health, Education, and Welfare, 1962). Because cyanide not infrequently is found in waterways due to industrial effluents, some studies have been conducted on the effects of cyanide on fish. The toxicity of cyanide to fish varies with temperature, oxygen, mineral content, and the pH values of the water. It should be pointed out that most of the investigations influencing the determination of our standards have been acute studies. There are very few studies concerned with the long-term effects of cyanide on aquatic life. Since cyanide concentrations in the range of 0.03 ppm have been reported to be lethal to fish, it is reasonable to expect that lower concentrations of cyanide probably would elicit some toxic manifestations in fish. It should be mentioned that in studies on aquatic invertebrates, 0.014 ppm is lethal to bivalve larvae. Also, the survival time of *G. pulex* is greatly shortened by 0.003 ppm of sodium cyanide. These data should generate some concern about the paucity of information available on effects of cyanide on aquatic life both in freshwater and seawater. The information which is available should be further evaluated. It is also important to recognize that chronic studies are essential to determine the long-term effects of cyanide on the life cycles of these various aquatic invertebrates and vertebrates. It should be emphasized that most of the acute studies focus on rapid lethal effects as the end point of cyanide toxicity. If one looks at more subtle cyanide effects, such as behavioral effects on fish, then the sensitivity of organisms to cyanide becomes more apparent. It has been reported that 10 μg/liter of cyanide (0.01 ppm) will impair the swimming performance of salmonid fish. In chronic studies performed on brook trout the following parameters were monitored: growth and survival of adults and spawning characteristics (i.e., number of eggs per female and egg viability, growth of embryos and juveniles, and embryo and juvenile survival). In these studies the maximum acceptable toxic concentration (MAT) of hydrogen cyanide was estimated to be between 6.0 and 11 μg/liter (0.006 to 0.011 ppm). Further studies conducted using the fathead minnow gave similar results. It would seem that chronic studies would provide a more valid rationale for setting standards for concentrations of various toxicants, such as cyanide in our waterways.

8.3.2 Terrestrial Organisms

The group of animals exposed most frequently to the threat of cyanide poisoning by the ingestion of cyanogenic plants is range animals, particularly cattle and sheep which graze on forage with a high cyanogenic glycoside content. In ruminants, the microorganisms in the rumen would hydrolyze the cyanogenic glycosides, liberating hydrogen cyanide. As pointed out earlier, this is most apt to occur during periods of drought, as the selectivity of forage by range animals becomes less discriminating. It is also believed that the cyanogenic glycoside content increases in some plants under dry climatic conditions. Under these conditions one would expect that livestock would be most apt to develop chronic as well as acute toxicity symptoms. There have been reports of possible chronic cyanide toxicity in horses; however, attempts to relate a well-defined toxin to symptoms of poisoning observed under field conditions are rather tenuous, as the effects can be ascribed to other factors present in the forage in addition to cyanide. For example, the syndrome described in the horse could be ascribed to lathyrogenic factors present in the forage rather than to cyanide itself. Another deleterious effect on livestock that results from grazing on forage which has a high cyanogenic glycoside content is thyroid dysfunction. This is attributed more to the formation of the metabolite of cyanide, thiocyanate, rather than to cyanide itself. It is of interest to note that various wild herbivores, such as deer and elk, have been observed to graze not infrequently on forage which contains a high content of the cyanogenic glycosides; yet, cyanide poisoning in wildlife has not been reported to any appreciable extent.

There have been some studies on the effect of cyanide on birds; however, they have been quite limited. Early studies on sparrows and pigeons have led to the general inference that birds are more susceptible to cyanide than mammals. Whether this impression is warranted or not is difficult to assess due to the limited studies conducted. The effects of cyanide on a variety of mammals have been studied in great detail. There is not uniform consensus that the dog may be more sensitive to cyanide than other species. Some investigators have noted that dogs have a lower rhodanese content than other animals and have ascribed this greater sensitivity to cyanide to a depressed detoxification mechanism. One should interpret the cyanide toxicity data in mammals with some degree of caution because the variation in susceptibility to cyanide from one species to another depends on many factors such as the route of administration and ambient temperature.

8.4 EFFECTS ON HUMAN HEALTH

8.4.1 Toxic Effects

The acute toxic effects of cyanide are well recognized. Cyanide intoxication can occur by exposure to the gas, the liquid, or inorganic salts. Hydrogen cyanide can be easily absorbed by inhalation as well as by topical and oral routes of administration. The rapidity with which cyanide exerts its lethal effect is well recognized by the general population.

Suspected chronic cyanide poisoning has been described and is alleged to be a rather serious and debilitating intoxication. Low-level chronic cyanide poisoning in man is difficult to document clearly in many cases since a variety of other causes can contribute to the signs and symptoms observed. Chronic poisoning may be occurring more frequently than is presently realized. Under industrial conditions where the cyanide is usually inhaled, a variety of signs and symptoms have been grouped to form what can be presently described as a "cyanide syndrome." These toxic effects involve the gastrointestinal tract, thyroid, and central nervous system. Other purported cases of chronic cyanide intoxication from the ingestion of cyanogenic foods have been reported. The syndrome of this tropical neuropathy is characterized by affliction of the nervous system producing optic atrophy, nerve deafness, and various types of ataxia. Demyelinization of fiber tracts in the central nervous system and peripheral nerves with resultant decreased conduction velocity has been described. In many of these studies, it is difficult to unequivocally ascribe the human nerve disorder specifically to cyanide; however, in some cases of tobacco amblyopia, hydroxocobalamin is effective in improving vision, providing rather convincing evidence that the lesion is more apt to be ascribed to cyanide exposure.

It should be noted that low chronic doses of cyanide have been reported to produce neuropathic lesions in man; however, attempts to experimentally produce neuropathic lesions in laboratory animals still require rather high doses of cyanide.

8.4.2 Teratogenic, Mutagenic, and Carcinogenic Effects

There have been no detailed studies which implicate cyanide as a teratogenic, mutagenic, or carcinogenic agent. There are also no well-documented studies employing controlled low-level chronic exposure of cyanide. Further studies along these lines are warranted, as cyanide is a very reactive nucleophile which should distribute rather widely through body compartments, should be permeable to cell membrane, and should attain reasonable concentrations in the fetus. It is of interest to note that cyanide has been reported to have antineoplastic properties and that it has been employed in clinical trials on humans. Epidemiological comparisons attempting to link cyanide with decreases in the incidence of tumors in the human population have not been very convincing.

8.4.3 Treatment

The time-honored basis for the treatment of cyanide poisoning proposed by Dr. K. K. Chen over 40 years ago involves a combination of drugs to bind and to detoxify cyanide. This therapeutic combination employed sodium nitrite to form methemoglobin, which would combine with cyanide to form cyanmethemoglobin, and sodium thiosulfate, which serves as a substrate for the enzyme, rhodanese, to convert cyanide to thiocyanate. More recently, oxygen was proposed as an integral part rather than as an adjunct therapy to this antidotal combination, as it strikingly enhances the protection against cyanide poisoning. This therapeutic regimen has protected against 20 LD_{50} doses of cyanide. Recently, some have advocated that intensive supportive medical care with special attention to pulmonary function may be of importance in treating cyanide poisoning. It would be of interest to determine whether such concepts as ventilated control and use of diuretic agents would contribute significantly to the treatment of cyanide poisoning.

Although the antidotal combination of sodium nitrite and sodium thiosulfate is used rather extensively in the United States, the use of cobalt EDTA is more prevalent in Europe, particularly in England and the

Scandinavian countries. The rationale for the use of cobalt is that it will form a stable metal complex with cyanide, thereby preventing its toxic effect. It seems more reasonable that the older conceptual approach to treat cyanide intoxication would be more efficacious (i.e., to bind cyanide as well as to detoxify it). If cobalt EDTA is to be employed, then it would seem that a more efficacious antidotal combustion would be to use cobalt EDTA in combination with sodium thiosulfate. More recently, new effective cyanide antidotes are being reported, and surprisingly, some of these antidotes neither combine nor detoxify cyanide.

8.5 POTENTIAL HEALTH HAZARDS

8.5.1 Occupational

Since cyanide is widely used in large amounts in various industries, this will remain a potential health hazard to workers, particularly because of the volatility of hydrogen cyanide and its ability to be absorbed by the inhalation and cutaneous routes. The U.S. standards proposed are to establish concentrations where no employee will suffer impaired health, functional capacity, or diminished life expectancy as a result of the work experience. The proposed standards developed by the National Institute of Occupational Safety and Health (NIOSH) apply to the processing, manufacturing, and use of hydrogen cyanide and its salt or their release as intermediates, by-products, or impurities as practicable under the Occupational Safety and Health Act of 1970. These standards were developed for general occupational exposure and should not be extrapolated to the general population. Both the federal standard and the American Conference of Governmental Industrial Hygenists (ACGIH) adopted threshold limit values (TLV) for hydrogen cyanide and alkali cyanide which greatly exceed the TLV instituted in some countries. It should be pointed out that the past recommendations by NIOSH are 8-hr time-weighted averages. The present federal standards are probably too high to provide adequate protection from the systemic effects of hydrogen cyanide and prevent the erosional effects on the nasal septum produced by the alkalinity of the cyanide salts.

8.5.2 General Population

There are potential health hazards to the general public from exposure to hydrogen cyanide and its salts. In countries where cassava is eaten as the major staple food, the high cyanogenic glycoside content presents a potential chronic health hazard to the general population. Because of the increased use of plastics in homes, liberation of hydrogen cyanide in home fires upon combustion of the plastics is coming under closer scrutiny in some countries. With regard to other sources of exposure to hydrogen cyanide on a long-term low-level basis, malfunctioning catalytic converters in automobiles do emit hydrogen cyanide; however, even under the most adverse conditions, projected on freeways, the general consensus is that this source alone would not be considered to be "hazardous" to the general public. The more likely concern to the general population would be restricted to those who reside near the electroplating, steel, mining, and paint manufacturing industries. Although cyanides can be released into the atmosphere, they are more likely to be released into the waterways. A recent incident of massive continuous outpouring of industrial effluent containing cyanide into waterways (Section 8.1.4.2.1) is of considerable concern. A more reliable system to continuously monitor industrial effluents containing cyanide needs to be instituted.

8.6 POTENTIAL ENVIRONMENTAL HAZARDS

Cyanide has been shown to be potentially toxic to microorganisms, aquatic invertebrates and vertebrates, and terrestrial animals. Sufficiently high concentrations of cyanide have provided a disruptive influence to the environment. Although hydrogen cyanide is volatile and birds have been reported to be extremely sensitive to cyanide, there is little information on avian poisoning as a result of the release of cyanide into the atmosphere.

Probably the greatest controllable sources of cyanide entry into the environment are industrial effluents in waterways. This can be a disruptive influence on the aquatic environment. Most studies to project recommended levels are based on acute lethal effects; however, investigations of the effects of long-term low-level dosages on aquatic organisms are essential. For example, the present allowable cyanide levels in U.S. waters could exert toxic effects on the life cycles of various fish. There is a paucity of information on the effects of chronic low levels of cyanide on the life cycle not only of fish but other aquatic organisms and microorganisms. The monitoring of the U.S. waterways indicating levels of less than 10 ppb does not necessarily imply that the problem of cyanide contamination is relatively minimal. Severe damage may result from the high pulsing of cyanide into waterways, which would not be detected by the present monitoring system. The factors which probably minimize the environmental hazard of cyanide are its chemical properties of volatility and reactivity. The pKa of cyanide indicates that it would be present predominately in the form of hydrogen cyanide, and therefore, its volatility upon agitation of the water would minimize its environmental persistence. Also, the biologic reactivity of cyanide should contribute to its rapid removal from aquatic systems.

Another environmental hazard which can be indirectly attributed to cyanide is the growth of various forage plants which have a high content of cyanogenic glycosides. The toxicity of this forage to livestock already has been discussed.

8.7 REGULATIONS AND STANDARDS

The American Conference of Governmental Industrial Hygenists (ACGIH) adopted a threshold limit value (TLV) for hydrogen cyanide on a time-weighted average of 10 ppm (11 mg/m^3). In 1971, the ACGIH TLV was indicated to present a twofold safety margin against mild symptoms and a sevenfold or eightfold margin against the lethal effects of cyanide. The present federal standard for hydrogen cyanide is also 10 ppm on a time-weighted average (29 CFR 1919.1,000 published in 39 FR 23541 on June 23, 1974) and is based on the 1962 ACGIH TLV. There are 13 foreign countries and 6 states in the United States that have set standards for hydrogen cyanide. It is difficult to compare these values as some countries use ceiling values, whereas other countries use time-weighted averages. It is of interest to note that five countries have set 0.3 mg/m^3 (0.27 ppm) as their standard, whereas the standards of most of the other countries are comparable with those of the United States. In the U.S. Department of Health, Education, and Welfare document (1976) on cyanide, it is recommended that employee exposure to hydrogen cyanide not exceed 5 mg/m^3, which would be determined at a ceiling concentration on a 10-min sampling period.

The ACGIH TLV for alkali cyanide is 5 mg/m^3 on a time-weighted average and the federal standard is identical (29 CFR 1919.1,000 published in 39 FR 23541 on June 27, 1974, as amended) and is based on the 1968 ACGIH TLV. It is of interest to note that there is a 17-fold difference in the standards between some foreign countries and the United States and that the United States has the higher TLV for alkali cyanide.

The federal standard and ACGIH TLV would protect against the acute toxicity from cyanide. However, with the cyanide salts the current federal standard of 5 mg/m^3 of cyanide on an 8-hr time-weighted average was recently suggested for revision by NIOSH, as it appeared to be too high and allowed for substantial excesses above that concentration for short time intervals. The U.S. Department of Health, Education, and Welfare document (1976) recommends that the same value of 5 mg/m^3 of cyanide be retained, but that the time base be changed from an 8-hr time-weighted average to a ceiling concentration on a 10-min sampling period. This was suggested to protect the workers from the systemic effects of cyanide and also to minimize the occurrence of nasal septum erosion produced by the alkalinity of the inorganic cyanide salts. These federal standards and threshold limit values may not have taken into account the low-level concentrations of cyanide which have been reported to produce chronic toxicity. It should be pointed out that 4.2 to 12.4 ppm has been reported to produce various signs and symptoms of chronic cyanide toxicity.

The recommended limit for cyanide in U.S. water is 10 ppb. Concentration in excess to 200 ppb constitutes ground for rejection of the water supply (U.S. Department of Health, Education, and Welfare, 1962). The U.S. Environmental Protection Agency recommended limit is 5 µg/liter. With regard to the effects of these limits on aquatic microorganisms and fish, it should be pointed out that, because cyanide lacks environmental persistence, the survey of U.S. public water supply systems revealing no cyanide concentrations above the mandatory limit is not reassuring, as high pulses of cyanide into waterways can cause profound changes in aquatic organisms and microorganisms without being detected by the methods presently employed.

In attempting to assess the effects of long-term exposure from the few studies conducted on occupational workers, the question always arises whether the signs and symptoms observed from purported chronic intoxication from cyanide may be attributed to other factors. However, the frequency of reported incidents of toxic manifestations resulting from exposure to low concentrations of cyanide has probably been sufficient to describe a "cyanide syndrome." The NIOSH recommendation of a lower exposure limit to hydrogen cyanide appears to be justified, particularly since these measurements were recommended to be conducted on a 10-min sampling period rather than an 8-hr time-weighted average. It should be pointed out that the USSR, Romania, Hungary, and Bulgaria recommend what appears to be ceiling concentrations of 0.3 mg/m^3 and Czechoslovakia recommends a standard of 3 mg/m^3 on a time-weighted average. Also in the United States, Hawaii recommends a standard of 20 ppm (approximately 22 mg/m^3) as a ceiling value. Pennsylvania also recommends this same standard as a 30-min ceiling value.

8.8 CYANIDE ANALYSIS

8.8.1 Biologic Sample

Analysis of cyanide is usually conducted using samples of whole blood. These samples should be obtained as soon as possible after cyanide exposure and analyzed immediately. Storage of blood samples can result in erroneous analytical values. It should be pointed out that variability in the temperature at which the samples are stored will contribute to variability in the final readings. Frozen blood samples may give lower values than those not frozen. Although most assays for cyanide are conducted using whole blood, there are some investigators who object to the use of whole blood and recommend the use of serum or plasma. However, in many cases these investigators may be unaware that the correlation among serum, plasma, and whole blood cyanide levels have been quite good at the higher cyanide concentrations.

8.8.2 Analytical Methods

The colorimetric method employing the König reaction with pyridine-pyrazolone reagent is one of the most widely used procedures for cyanide analysis. It is a sensitive and accurate method; however, it is subjected to interference by some anions. Many of the studies showing the efficacy of sodium thiosulfate in detoxifying cyanide by converting it to thiocyanate based upon this method of analyses may be erroneous. Sodium thiosulfate when acidified forms polythionic acids which volatilize, presumably as sulfur dioxide, and are trapped in the alkaline media as sulfite anion. It is this latter anion which will interfere with the colorimetric determination of cyanide. Therefore, the rapidly falling cyanide levels attributed to sodium thiosulfate administration in cases of cyanide poisoning may be erroneously low due to the anion interference with the colorimetric method rather than the presumed enhanced conversion of cyanide to thiocyanate. The more recent method for determining cyanide which employs ion-selective electrodes is quite sensitive. It is a much more convenient and rapid method for analyzing cyanide. Automation has been achieved employing these electrodes and commercial models are available.

Various fluorometric methods are available for the determination of cyanide. In general, the fluorometric methods are sensitive, but the results obtained are not as consistent as with the colorimetric method. One of the fluorometric methods has the advantage that it is not subjected to anionic interferences as with the pyridine-pyrazolone procedure. However, this specific fluorometric method does not give a linear response. Automation has also been obtained using a fluorometric method.

Gas chromatographic methods of measuring cyanide are usually based on its conversion to cyanogen chloride. This conversion is needed because most gas chromatographic detectors are not very sensitive to cyanide itself. The measurement of cyanide as cyanogen chloride is quite sensitive and it is surprising that this method is not more widely used.

8.9 SUMMARY OF OPINION AND PROJECTED RESEARCH NEEDS

The concentration of cyanide allowed in waterways should be reevaluated with some consideration directed toward the long-term effects of cyanide on life cycles, growth, and survival as well as biochemical, physiological, and behavioral effects. The present limits have been primarily based on acute toxicity studies where lethality is the end point. Investigations using fish clearly delineate the limitations of this type of data. Not only should chronic studies be conducted, but these studies should be conducted in a variety of aquatic organisms and microorganisms in addition to fish.

In an area where potential for entrance of cyanide into waterways exists, analysis should be conducted employing a continuous monitoring system rather than intermittent sampling. The environmental persistence of cyanide is probably quite low; therefore, periodic entrance of hydrogen cyanide into our waterways can promote considerable damage to aquatic life without being detected. Because of the rapid action, high toxicity, and low environmental persistence of cyanide, periodic monitoring of cyanide in waterways does not produce sufficient assurrance that an ecological hazard does not exist.

The potential formation of hydrogen cyanide in home fires warrants further studies. Pyrolysis of various plastics involved in home construction should be studied and the interaction of the various gases liberated should be investigated from a toxicological viewpoint.

Intensive low-level long-term studies on cyanide intoxication in mammals should be undertaken. These studies should include measurement of concentrations of cyanide, its metabolites, thyroid function, EEG, blood chemistry, and in the case of humans, medical history, occupation, smoking habits, history of potential exposure, and air samples in living and occupational areas. Controlled laboratory studies on the effects of chronic low-level administration of cyanide should be conducted. Substantial studies of effects of long-term low-level cyanide exposure by the oral and inhalation routes should be undertaken with particular attention focused on the neuropathic lesions which may be produced. These types of studies may provide a more valid rationale in establishing federal standards and threshold limit values for hydrogen cyanide and inorganic cyanide.

With respect to livestock, more studies are needed on the various factors, such as drought, which may increase the concentration of cyanogenic glycosides in plants.

There is a paucity of information on the teratogenic, mutagenic, and carcinogenic effects of cyanide at the acute, subacute, and chronic levels, and the information which is available should be further investigated.

Although the antidotal therapy of cyanide intoxication is quite efficacious, additional studies are warranted because of the high toxicity and rapid action of cyanide. These studies should focus on supportive medical therapy, better antidotes, and different antidotal combinations and should be conducted not only on laboratory rodents but on other species including range animals.

Epidemiological studies may be warranted in an attempt to correlate low-level long-term exposures of cyanide to various toxic manifestations. For example, in occupationally exposed workers and in the general population in vicinities of high cyanide concentrations, cyanide levels may be correlated with the incidence of skin dermatitis, nasal septum lesions, thyroid dysfunction, and urinary thiocyanate levels.

Continuous automatic monitoring equipment to measure cyanide in air and water should be encouraged and these devices should include automatic alarm systems when predetermined levels are exceeded.

Studies on the production of hydrogen cyanide under various conditions in malfunctioning catalytic converters should be further investigated. These studies should focus on those factors which can promote maximal formation of hydrogen cyanide and on its toxicity alone and in combination with other automobile emission gases in regard to possible potentiation of toxicity.

The effect of cyanide on wildlife and range animals, particularly those which could graze on foliage with a high cyanogenic glycoside content, warrants further studies.

The mechanism of uptake, metabolism, and biosynthesis of cyanide in soils, plants, and various fungi merits further investigation. At the present time there is little information on the mechanism of cyanide liberation by plants and microorganisms, and almost no information on their contribution to total cyanide in the environment.

SECTION 8

REFERENCES

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